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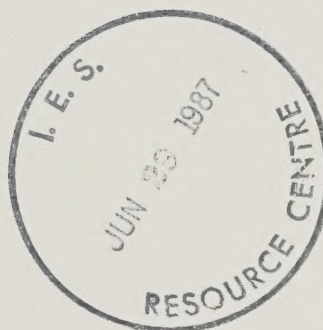
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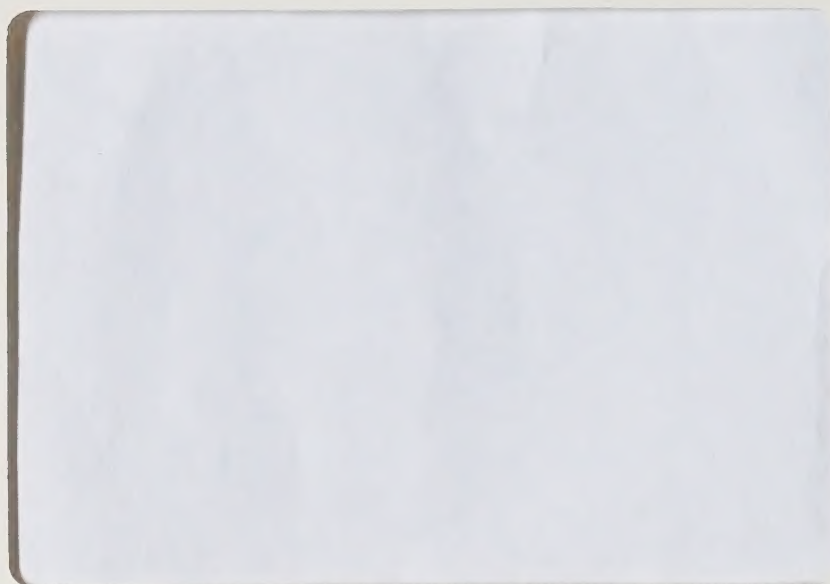
EXPERT PANEL REPORT ON
CARCINOGENICITY
OF 2,4-D

March 23, 1987



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PREFACE

This report was prepared at the request of the Health Protection Agency Committee of the Council Ministers of the Government. The panel of Experts was appointed by the Joint Japan-Canadian Minister of the Environment to address the following areas of concern.

To address the scientific and health significance of existing epidemiological and environmental data on the carcinogenicity of 2,4-D and its metabolites, on the basis of the

EXPERT PANEL REPORT ON CARCINOGENICITY OF 2,4-D

March 23, 1987

The report of the Expert Panel was reviewed by a joint staff of the Department of Pathology, Pharmacology and Physiology, University of Toronto.

Canadian Centre for Toxicology,
Guelph, Ontario, Canada





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PREFACE

This report was prepared at the request of the Ontario Pesticides Advisory Committee of the Ontario Ministry of the Environment. The Panel of Experts was appointed by the Hon. James Bradley, Minister of the Environment to address the following terms of reference:

To assess the validity and health significance of existing experimental and epidemiological data on the carcinogenicity of 2,4-D and to determine, on the basis of the existing data on carcinogenicity, whether any of the existing uses of 2,4-D in Ontario pose a significant health risk.

The Expert Panel was chaired by Dr. I.C. Munro of the Canadian Centre for Toxicology with resources supplied by the Centre.

The report of the Expert Panel was reviewed by Dr John Doull of the Department of Pharmacology, Toxicology and Therapeutics, University of Kansas.

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1. SUMMARY AND EVALUATION

The herbicide, 2,4-Dichlorophenoxyacetic acid (2,4-D) was introduced commercially in Canada during the 1940's. It is widely used as a herbicide in forestry, agriculture, turf maintenance and for weed control in parks and rights-of-way. It has extensive applications in the home and garden market as well. Several formulations of 2,4-D are registered for use in Canada, normally as the amine salt or as esters of the acid. The total volume of 2,4-D sold in Ontario during 1986 was approximately 500,000 kg active ingredient.

Concern over the safety of 2,4-D first arose in the 1960's when it was recognized that certain 2,4-D formulations were contaminated with dioxins. Although chlorinated dioxins have been identified as contaminants in 2,4-D products and formulations, current Canadian regulations allow only low concentrations (<10 ug/L) to be present. Moreover, the highly toxic 2,3,7,8-tetrachlorodioxin has not been identified, nor would it be expected in 2,4-D products and formulations. The dioxins that have been identified in 2,4-D products and formulations (2,7- or 2,8-dichlorodioxin, 1,3,7- or 1,3,8-trichlorodioxin, and 1,3,6,8- or 1,3,6,9-tetrachlorodioxin) are not considered highly toxic (Ontario Ministry of the Environment, 1985).

N-nitrosodimethylamine, a carcinogenic nitrosamine, also has been detected at low levels in certain samples of commercial 2,4-D particularly when nitrite was added as a rust inhibitor. The levels detected have ranged from 0.3-5 mg/L in the formulated product. A risk assessment on nitrosamines in 2,4-D conducted by the U.S. National Academy of Sciences indicated that the amounts found in 2,4-D formulations pose, at most, a negligible risk to human health.

Pharmacokinetic studies conducted on 2,4-D in experimental animals and humans indicate that 2,4-D is absorbed via all routes of exposure. It appears that 2,4-D is distributed widely among the tissue of the body; the highest concentrations are found in parenchymal and excretory organs. There is no evidence that 2,4-D is metabolized to reactive intermediates which might bind to tissue macromolecules such as DNA. In all mammals examined, including humans, 2,4-D is rapidly excreted in the urine, largely unchanged in chemical form, though a small but variable amount may be conjugated in the kidney prior to excretion. These conjugated forms, which are highly polar and rapidly excreted, would be expected to be even less toxic than 2,4-D.

The available pharmacokinetic studies indicate that there is a proportional and constant relationship between exposure, uptake and urinary elimination of

2,4-D in workers exposed over several days and who have achieved steady state pharmacokinetics. These studies further indicate that, in workers who use 2,4-D regularly, the amount excreted in the urine over a 24-hour period is a reliable measure of the absorbed systemic dose. This finding facilitates the measurement of dose received by workers employed in occupations involving 2,4-D exposure.

Exposure studies have been conducted on 2,4-D in Ontario or in areas with similar climate and use pattern. These indicate that hydro-line workers may be exposed to the 2,4-D in amounts from 0.005 - 5 mg/person/spraying day. Exposure in this setting is highly variable and depends upon the nature of the work performed (mixer-loader, sprayer, flagger, etc.) and the extent to which precautionary procedures are followed and protective gear is worn. Commercial lawn applicators were found to receive a daily dose of approximately 0.3 mg/person/spraying day while farmers were estimated to receive about 0.5 mg/person/spraying day. Variation between these groups is due to differences in spray equipment, terrain and to the degree to which the operators come into contact with concentrated formulations. The latter is the most probable reason for the low levels of exposure in commercial lawn applicators who were reported to take appropriate precautions when mixing the formulation.

Genotoxicity studies on 2,4-D have included in vitro studies in bacteria, yeasts and cultured mammalian cells. In vivo genotoxicity studies have been conducted in rats, mice, hamsters and Drosophila. In addition, some limited studies have been carried out in humans exposed occupationally to 2,4-D.

The results of tests conducted in in vitro systems indicate that 2,4-D is not mutagenic in Salmonella or E. coli; however, some conflicting data have been reported in B. subtilis. This test correlates poorly, if at all, with carcinogenicity. There is an isolated report that 2,4-D "fluid" induced unscheduled DNA synthesis in human fibroblasts; however, no such effects were noted in more definitive studies in human embryonic lung cells and cultured rat hepatocytes. No information was given on the composition of the 2,4-D "fluid" and thus the significance of this positive report cannot be evaluated.

It has been reported that 2,4-D induced mutations in yeast but positive effects were noted only below pH 4.5, leading the authors of these studies to conclude that effects were dependent entirely on pH of the culture media.

There are studies indicating that 2,4-D produces sister chromatid exchanges (SCE's) in cultured human lymphocytes but not in hamster embryo cells.

The significance of these findings is questionable in light of the fact that several in vivo studies involving rats, mice, hamsters and humans have not shown any effects on SCE's in lymphocytes or bone marrow cells when 2,4-D was administered by appropriate routes at up to toxic doses. In addition, SCE's in vitro cannot be viewed as reliable predictors of carcinogenicity. There is one report that 2,4-D induced chromosomal aberrations in mouse bone marrow cells; however, the significance of this finding is questionable because the animals were given a dose corresponding to the LD₅₀.

Conflicting data exist on the mutagenic activity of 2,4-D in Drosophila. Unstable strains appear to show weakly positive effects while more stable strains appear to be resistant even at very high dietary concentrations (e.g. 1,000 ppm). A micronucleus test and a dominant lethal assay conducted in mice at doses of 100-125 mg/kg produced negative results.

In summary, in vitro studies on the genotoxicity of 2,4-D, in some cases, produced conflicting results, however, there is no convincing evidence that 2,4-D produces mutagenic effects when it is tested in in vivo systems. Overall, the pattern of responses observed in both in vitro and in vivo tests indicates that 2,4-D is not genotoxic.

The carcinogenicity of 2,4-D has been studied in two recently completed, long-term cancer bioassays, conducted in the United States under the auspices of the Industry Task Force on 2,4-D Research Data. In one study, groups of male and female Fischer 344 rats were given 0, 1, 5, 15, or 45 mg 2,4-D(acid, 97.5% pure)/kg body weight/day for 2 years. The results of this study indicated an increased incidence of brain tumours (astrocytomas) in male rats of the high-dose group. No treatment-related increase in brain tumours was noted in female rats or at any other site in either males or females.

The incidence of brain tumours in male rats treated at the high dose was significantly increased compared to concurrent untreated controls. The incidence in the high dose group also exceeded that of tumors observed historically in untreated male rats of the same strain.

While it is not possible to discount this evidence for carcinogenesis, the characteristics generally attributed to a brain carcinogen were not present in this experiment. There was no evidence of decreased tumor latency, the increase was limited to high-dose males, no preneoplastic lesions such as gliosis were present in treated animals, all tumors were solitary, and the tumors in treated animals were not more advanced (anaplastic) than generally seen in control animals.

Considering this, the Panel concludes that there is insufficient evidence to be certain that the brain tumors were related to 2,4-D exposure. This conclusion is supported by the large body of negative genotoxicity data on 2,4-D. In addition, there is no evidence to indicate that 2,4-D forms reactive intermediates in the liver or other tissues or forms adducts with DNA.

In the industry-sponsored 2,4-D mouse study, groups of male and female B6C3F1 mice were treated with the compound at dose levels of 0, 1, 15 or 45 mg 2,4-D(acid, 95% pure)/kg body weight/day in the diet for 106 weeks. The Panel considered that, in this study, a higher maximum dose could have been used; however, the highest dose used exceeded the highest estimated average occupational exposure by a factor of about 600. The results of this study did not indicate any relationship between 2,4-D exposure and tumour incidence in either male or female mice.

Epidemiological studies conducted on phenoxy herbicide-exposed workers have involved both case-control and cohort type studies. In one series of case control studies conducted in Sweden, an increased relative risk of soft tissue sarcoma and malignant lymphoma from exposure to phenoxy herbicides was noted; however, this was not found in similar studies conducted in New Zealand. In a cohort study in Denmark, however, an excess of soft tissue sarcomas in phenoxy-exposed workers was found. Several other cohort studies have been negative. An excess of Non-Hodgkins Lymphoma, but not soft-tissue sarcoma was observed in a case-control study conducted on herbicide-exposed farmers in Kansas.

Based on the available epidemiological studies 2,4-D cannot be exonerated as a reason for the excess cancer risk seen in studies involving the phenoxy herbicides conducted in the U.S., Denmark and Sweden, but neither can these studies identify 2,4-D as being the causative agent. Overall, the epidemiological evidence indicates that a relationship between an increased risk of soft-tissue sarcoma and non-Hodgkins lymphoma with phenoxy herbicide exposure is tenable; however, in regard specifically to 2,4-D, the evidence for human carcinogenicity must be considered as inadequate.

The evaluation of the validity and health significance of existing data pertaining to the carcinogenicity of 2,4-D is difficult. Because the epidemiological studies were conducted on persons exposed to several herbicides, it is not possible to identify the role, if any, of 2,4-D on the putative relationship between phenoxy herbicide exposure and increased risk of soft-tissue sarcoma and non-Hodgkins lymphoma. The epidemiological studies, by themselves,

cannot be used to assess the possible carcinogenicity of 2,4-D. Other studies of 2,4-D carcinogenicity have demonstrated an increased incidence of brain tumors in male rats given 2,4-D at 45 mg/kg body weight/day. However, as discussed above, there is insufficient evidence to conclude that the tumors were related to 2,4-D exposure. Overall, the Panel concludes that the existing animal and human data are insufficient to support the finding that 2,4-D is a carcinogen and, consequently, finds insufficient evidence to conclude that existing uses of 2,4-D in Ontario pose a significant human health risk.

2. INTRODUCTION

The chlorophenoxy herbicides were developed during the early 1940s and the compounds 2,4-dichlorophenoxyacetic acid (2,4-D,) 2,4,5- trichlorophenoxyacetic acid (2,4,5-T) and (2-chloro-2-methylphenoxyacetic acid (MCPA) were commercially introduced in the mid 1940s. By the mid 1960s, the chlorophenoxy herbicides were the most widely used class of herbicides. They are generally formulated either as the amine salts or as esters, the latter commonly of isooctyl and butoxyethanol alcohols. As a herbicide, 2,4-D is used for the selective control of a large number of broad-leaf weeds in cereals, grains and turf crops as well as the control of shrubs, broad-leaf weeds and trees in rangeland, forests and rights-of-way. Rates of application may vary from 0.25 kg/ha in grain crops to 16 kg/ha in spot treatment of trees and bushes in rights-of-way. Average use of 2,4-D in the USA for the years 1978-1983 was 10 million kg (range 14.3-6.2 million kg) while use in Canada was reported to be 3.5 million kg for the years 1974-1976 (IARC, 1987). Total sales of 2,4-D during 1986 in Ontario were reported as 0.5 million kg (DHS, 1987).

Concern over the human health implications of exposure to the phenoxy herbicides focussed initially on 2,4,5-T in the late 1960s with the discovery that some formulations containing this chemical were contaminated with dioxins and, in particular, 2,3,7,8-tetrachloro-p-dibenzodioxin (2,3,7,8-TCDD). Restriction were placed on the use of 2,4,5-T in a number of jurisdictions (including Ontario) and it is now no longer manufactured in North America. This concern also resulted in what was probably the most thorough and extensive review of the scientific literature on the subject (Veterans Administration, 1981a and b; 1983: Royal Commission, 1985).

The recent discovery of dioxins in certain formulations of 2,4-D (Cochrane et al., 1981) raised concern for this product as well and prompted the establishment of regulations limiting dioxin levels in 2,4-D products in Canada. Recent epidemiological evidence, especially the so-called "Kansas study" (Hoar et al., 1986a and b) and the report that chronic feeding with 2,4-D increased the incidence of astrocytomas in male rats (Hazleton, 1986) have further raised the level of concern for the human health implications of this compound. These were the primary reasons for the commissioning of this report.

The terms of reference of the Expert Panel were as follows:

1. To assess the validity and health significance of existing experimental and epidemiological data on the carcinogenicity of 2,4-D.
2. To determine, on the basis of the existing data on carcinogenicity, whether any of the existing uses of 2,4-D in Ontario pose a significant health risk.

In light of these terms of reference, the panel has concentrated its efforts on the question of carcinogenicity and possible related effects. Only brief reference is made to other possible adverse effects.

3. ACUTE AND SUB-CHRONIC TOXICITY

The acute and sub-chronic toxicity of 2,4-D is well documented and was recently reviewed by the World Health Organization (WHO, 1984). A summary of the acute and sub-chronic toxicity data on 2,4-D is presented in Table 1.

The available evidence indicates that 2,4-D is of moderate acute toxicity to mammals and birds. Clinical signs of acute exposure to high doses of 2,4-D include effects on the gastrointestinal tract, muscular weakness, muscle spasms and depression of the central nervous system (CNS). CNS depression is thought to be due to alterations in the blood-brain barrier at very high exposure levels (Elo and Ylitalo, 1977 and 1979). The myotoxic effects of acute exposure to 2,4-D are reported to include changes in a number of physiological and biochemical processes in muscle (WHO, 1984). Direct myotoxic effects of 2,4-D may have been mistakenly interpreted as symptoms of peripheral neuropathy in some animals and WHO (1984) stated that the existing data are inadequate to assess the possible role of 2,4-D in the development of peripheral neurotoxicity. More recent studies have not reported any evidence of peripheral neurotoxicity. Mattsson et al. (1986a) did not find peripheral neuropathy in male and female Fischer 344 rats treated dermally with 12% 2,4-D amine for two hours daily, five days a week for three weeks (equivalent to ca. 110 mg 2,4-D acid/animal/day). A similar lack of effect was also noted in male Fischer 344 rats treated with 24% 2,4-D amine for two weeks in the same manner (Mattsson et al., 1986b).

There have been some reports of peripheral neuropathy attributed to 2,4-D exposure in humans. Symptoms associated with exposure to phenoxy herbicides were said to include: Reduced peripheral nerve conduction velocity, long-lasting flacid paraparesis (incomplete paralysis) or quadriparesis, abnormal

tendon reflexes, sensory neuropathy. The relationship between 2,4-D exposure and peripheral neuropathy in humans has been questioned. No indications of similar effects were reported in persons exposed to "massive" exposure to 2,4-D or in patients given pure 2,4-D or 2,4,5-T (and their esters) as drugs (WHO, 1984). It is possible that the effects reported were associated with exposure to other agents such as solvents, nutritional or hereditary conditions, infections and alcoholism and it has been suggested that further studies should be carried out using more modern methods (WHO, 1984).

Table 1. Acute and chronic toxicity of 2,4-D in animals.

Compound/ formulation	Species	Sex	LD ₅₀ or criterion
<u>ORAL TOXICITY</u>			
2,4-D acid	Mouse	M	375-368 mg/kg body weight
	Rat	M	375-666
	Guinea-pig	MF	469-1000
	Rabbit	NA	800
	Dog	NA	100
	Chicken	NA	541
Various ester formulations	Mouse	MF	380-570
	Rat	MF	620-1500
	Guinea-pig	MF	550-848
	Cat	NA	820
	Rabbit	MF	1420
	Chicken	NA	900-2960
Sodium salt	Mouse	NA	375
	Rat	F	805-2000
	Guinea-pig	M	551
	Rabbit	NA	800
	Chicken	F	655
<u>SUB-CHRONIC STUDIES</u>			
Acid or sodium salt	Rat	NA	31 mg/kg body weight/day NOEL.
<u>REPRODUCTIVE EFFECTS</u>			
Acid or sodium salt	Mammals and birds	NA	10 mg/kg body weight/day NOEL for teratogenic, fetotoxic or embryotoxic effects.

Source: WHO, 1984

3.1. Contaminants in 2,4-D

3.1.1. Polychlorinated dibenzo-p-dioxins

Although Norström *et al.* (1979) reported that no polychlorinated dibenzo-p-dioxins were detected (detection limits were 0.01 to 0.05 mg/L for di- to hexachloro isomers) in samples of 2,4-D and 2,4-D ester produced in 1965 or

earlier, one sample contained 0.06 mg/L of pentachlorodibenzofuran. He also reported that no di-, tri-, penta-, or hexachlorodibenzofurans were detected.

The presence of dioxins in 2,4-D was first reported by Cochrane *et al* (1981). Samples of technical and formulated products containing 2,4-D esters and amines were analyzed by gas chromatography/mass spectrometry. Isooctyl formulations of 2,4-D contained 2,7- or 2,8-dichlorodioxin in concentrations ranging from 104 to 4,200 ug/L. Some isooctyl ester samples also contained 1,3,7- or 1,3,8-trichlorodioxin (346 to 2,079 ug/L) and 1,3,6,8-tetrachlorodioxin (226 to 1,752 ug/L). Two of three samples of 2,4-D mixed butyl esters and four of seven samples of 2,4-D dimethylamine salts showed the presence of dioxins.

A later study of dioxin contaminants, conducted after regulations of dioxin contamination had been promulgated (limiting the levels of dioxins to less than 10 ug/kg), revealed low dioxin concentrations (Cochrane *et al*, 1982). Of one hundred and ninety-nine samples that were analyzed, only 0/72, 7/78, and 2/49 samples of 2,4-D acid, amines, and esters, respectively exceeded the regulatory limit of 10 ug/kg.

The highly toxic 2,3,7,8-tetrachlorodioxin has not been identified and nor would it be expected in 2,4-D products and formulations. The toxicity of the dioxins identified in 2,4-D products and formulations (2,7- or 2,8-dichlorodioxin, 1,3,7- or 1,3,8-trichlorodioxin, and 1,3,6,8- or 1,3,6,9-tetrachlorodioxin) has been recently reviewed and is considerably less than 2,3,7,8-TCDD (Ontario Ministry of the Environment, 1985).

3.1.2. Nitrosamines

Concentrations of N-nitrosodimethylamine (NDMA) in pesticide products have been determined (Cohen *et al.*, 1978). Samples of dimethylamine, which may be used in amine salt formulation, showed NDMA concentrations of 27.5 to 53 mg/L. The NDMA content of formulations of 2,4-D dimethylamine salts ranged from not detectable to 6 mg/L. Studies have revealed N-nitrosamine contamination (up to 0.3 mg/L) in amine formulations of 2,4-D, particularly when nitrite was added as a corrosion inhibitor (WHO, 1984). N-nitrosodimethylamine has also been detected in dimethylamine salts of 2,4-D in Canada (Reid, 1984). Of one hundred and twelve 2,4-D samples analyzed, ninety-two, sixteen, and four samples contained ≤ 1 mg/L, 1 to 5 mg/L, and ≥ 5 mg/L N-nitrosodimethylamine, respectively.

Based on a risk assessment conducted by the National Academy of Sciences (NAS, 1981), and the exposures estimated in this document, the amounts of N-nitrosodimethylamine in 2,4-D pose, at most, a negligible risk.

4. PHARMACOKINETICS AND METABOLISM OF 2,4-D IN HUMANS AND EXPERIMENTAL ANIMALS

The absorption, distribution kinetics and metabolism of 2,4-D (in a variety of formulations) has been extensively reviewed (WHO, 1984) with the general conclusion that 2,4-D is not significantly metabolized in animals although a certain proportion of the absorbed dose may be conjugated prior to excretion. These studies are pivotal to the assessment of human exposure and are reviewed in more detail below.

4.1. Animal studies

The distribution and elimination of orally administered 2,4-D amine (commercial formulation, triethanolamine salt), 2,4-D K-Na salt ("pure acid"), and 2,4-D ester (commercial formulation, butyl ester) were studied in rats, pigs, calves, chicks, and chickens (Erne, 1966a and b). After single oral doses of 2,4-D amine, amounting to 50, 100, or 200 mg 2,4-D/kg, peak plasma concentration was reached within 2 h after dosing in chickens and 4 to 7 h after dosing in the mammalian species studied. Plasma half-life of 2,4-D after a single oral dose of 100 mg/kg was determined in several species:

<u>Compound</u>	<u>Species</u>	<u>Half-life, h</u>
2,4-D amine	Rat, male	2.9 $\pm$ 0.4
"	Rat, female	3.3 $\pm$ 0.5
"	Pig	12 $\pm$ 2
"	Calf	7.5 $\pm$ 0.8
"	Chicken	7.7 $\pm$ 0.7
2,4-D K-Na	Rat, male	3.5 $\pm$ 0.5
"	Calf	8.0 $\pm$ 0.6
2,4-D ester	Rat, male	6 $\pm$ 1
"	Pig	10 $\pm$ 0.8
"	Calf	10 $\pm$ 1

Repeated oral doses of 50 mg/kg/day 2,4-D amine and ester were given to pigs. In most animals, plasma 2,4-D concentrations declined steadily, and no evidence of accumulation was seen; however, in one pig given 2,4-D amine and in another pig given 2,4-D ester, plasma 2,4-D concentrations rose, and both animals showed signs of intoxication. Tissue 2,4-D concentrations were also measured after giving a single oral dose of 2,4-D. In general, tissue 2,4-D concentrations were highest in parenchymal organs (liver, kidney, lung). In

male rats given 2,4-D amine (100 mg 2,4-D/kg), plasma concentrations were 150 and 2 ug/g at 6 and 24 h after dosing; liver concentrations 90 and 5 ug/g at 6 and 24 h; kidney concentrations 250 and 27 ug/g at 6 and 24 h. In pigs given 2,4-D amine (100 mg 2,4-D/kg) orally, plasma 2,4-D concentrations at 6, 24, 48 and 72 h after dosing were 210, 55, 10 and 3 ug/g; brain concentrations at 6, 24, and 48 h after dosing were 12, 3, and 1.5 ug/g. In chickens given 2,4-D amine (200 mg 2,4-D/kg) the plasma and brain concentrations 6 h after dosing were 100 and 1.5 ug/g, respectively. In pigs fed 500 ppm 2,4-D amine in their feed for 2 months, plasma, liver, kidney and brain 2,4-D concentrations were 22, 6, 12 and 2 ug/g respectively. It was found that 0 to 18% of the 2,4-D present in urine after giving single or repeated oral doses of 2,4-D amine was present as an acid-hydrolyzable conjugate the structure of which was not identified. The ester of 2,4-D was found to undergo very rapid hydrolysis to 2,4-D (Erne, 1966a and b).

Metabolism of orally administered [^{14}C]2,4-D has been studied in rats (Khanna and Fang, 1966). Radiolabelled carbon dioxide was not detected as a metabolite of 2,4-D. In rats given 1 to 10 mg 2,4-D per rat (males: 350 to 400 g; females: 225 to 275g body weight), 93 to 99% of the administered radioactivity was excreted in the urine in 48 h. When 20 to 100 mg 2,4-D/rat was given, a smaller fraction of the dose was recovered in the urine over a 12 day period; i.e.:

Dose/rat (mg 2,4-D)	20	40	60	80	100
Recovery in urine (%)	91.3	87.1	87.4	77.9	75.5

In rats given 1 mg 2,4-D/rat, the highest brain concentration of 0.7 ug/g dry tissue was found 6 h after dosing. In rats given 80 mg 2,4-D/rat, the following concentrations were found:

Hours after treatment	4	8	24	41
Blood concentration (ug/g)	414	805	268	7
Brain concentration (ug/g)	20	66	16	1

The presence of a urinary metabolite of 2,4-D, which accounted for 0.25% of the administered radioactivity, was detected, but not identified. No evidence for the incorporation of 2,4-D or metabolites into tissue constituents was found.

In male rats dosed orally with 2,4-D (200 mg/kg; 97.9% pure dissolved in peanut oil), a total of 1.4% of the dose was eliminated as a glycine conjugate, and 1.4% as a taurine conjugate (Grunow and Böhme, 1974).

In studies designed to evaluate the effects of 2,4-D intoxication on the distribution of 2,4-D, adult male Sprague-Dawley rats (200 to 290 g) were given 0 or 250 mg 2,4-D/kg (sodium salt, 97% pure) by subcutaneous injection (Elo and Ylitalo, 1977; 1979). Three and a half to 4.5 h after treatment, the rats were given 8.8 mg of [^{14}C]2,4-D (98% pure) intravenously. Starting 30 min. later, the cerebrospinal fluid was collected for 1 h. Plasma and tissue samples were collected immediately after the termination of the cerebrospinal fluid collection period. Although absolute plasma or tissue 2,4-D concentrations were not reported, in saline-treated controls the brain and cerebrospinal fluid values amounted to 2.3 and 0.4% respectively, of the plasma values; after intoxication with 250 mg 2,4-D/kg, the brain and cerebrospinal fluid values rose to 15 and 9.4% of the plasma values. The administration of 250 mg 2,4-D/kg increased brain and cerebrospinal fluid tissue/plasma ratios of ^{14}C 6.5- and 23.5-fold, respectively; smaller increases, which amounted to 1.6 to 3.3-fold, were seen in liver, testis, lung, heart, and muscle tissue. The authors concluded that 2,4-D intoxication either increases the influx of 2,4-D into the brain or decreases the efflux of 2,4-D out of the brain.

Male Fischer 344 rats were given single oral doses of 10, 50 or 150 mg or intravenous doses of 5 or 90 mg ring labelled [^{14}C]2,4-D (>99% radiochemical purity) and plasma and urine content of 2,4-D measured for 72 h (Smith *et al.*, 1980). The rate constant for absorption of orally administered 2,4-D was 1.4 per hour and it was rapidly cleared from the blood in a biphasic manner: The $t_{1/2}$ (alpha) for the plasma clearance of intravenously and orally administered 2,4-D was 0.92 and 1.01 h respectively; the $t_{1/2}$ (beta) for the plasma clearance of intravenously and orally administered 2,4-D was 14.4 and 18.0 h, respectively. At doses >50 mg 2,4-D/kg there was a disproportionate increase in plasma ^{14}C concentrations and a decrease in the ^{14}C content of urine. The K_m for the saturable clearance of 2,4-D from the plasma was about 79 ug/ml: at concentrations below the K_m , plasma clearance of 2,4-D follows first-order kinetics. The authors conclude that the saturable clearance of 2,4-D is attributable to the saturable urinary excretion of 2,4-D.

The pharmacokinetics of dermally administered ring labelled [^{14}C]2,4-D propylene glycol butyl ether ester (2,4-D PGBE ester; 97.6% chemically pure; 99.4% radiochemically pure) was studied in rats (Smith *et al.*, 1981). The ester

(5 mg/kg) was applied in a single dermal application of an acetone solution to male Fischer rats and elimination of ^{14}C studied for 120 h after treatment. Absorption of 2,4-D PGBE ester through the skin followed first-order kinetics ($t_{1/2} = 19.7$ h) and an average of 85% of the applied radioactivity was recovered in the urine after 120 h. The authors concluded that the pharmacokinetics of 2,4-D PGBE ester are similar to that of 2,4-D acid.

Frantz and Kropscott (1984) studied the pharmacokinetics of the 2-ethylhexyl ester in rats. Male and female Fischer 344 rats were given a single oral dose of 130 mg 2,4-D (2-ethylhexyl ester) in corn oil, and blood and urine sampled for 72 h. The ethylhexyl ester could not be detected in blood or urine for 72 h after treatment, although 2,4-D was present in both blood and urine. A total of $94.8 \pm 9.2\%$ and $84.3 \pm 4.5\%$ of the dose was recovered in the urine of male and female rats, respectively, in 72 h. The authors concluded that 2,4-D 2-ethylhexyl ester is very rapidly hydrolyzed to 2,4-D, which is excreted in the urine.

Similar pharmacokinetic studies of 2,4-D have also been conducted in the mouse (Hazleton Laboratories, 1984). Male B6C3F1 mice were given 5, 45 or 90 mg orally as an aqueous solution or 90 mg ring labelled [^{14}C]2,4-D (98% pure) intravenously. Urinary and fecal elimination of ^{14}C was measured for 168 h after dosing. Urinary excretion of 2,4-D amounted to 53 to 71% of the dose; fecal excretion of 2,4-D to 5 - 15% of the dose and a greater fraction of the ^{14}C was found in the feces of mice in the higher dose groups. The $t_{1/2}$ for absorption of 2,4-D from the gut was approximately 0.013 h. K_m and $V_{\max}$ for the elimination of 2,4-D were dose dependent, i.e.:

Dose (mg/kg)	5	45	90
K_m	4.5	15.4	58.0
$V_{\max}$	7.3	20.7	28.2

The authors concluded that the elimination of 2,4-D in the mouse was dose dependent and deviated from classical Michaelis-Menten kinetics.

In summary, results of animal studies on 2,4-D absorption, distribution, excretion and pharmacokinetics indicate that 2,4-D, its salts and esters are well absorbed from the gastrointestinal tract and peak plasma concentrations are reached soon after dosing. In addition, 2,4-D is rapidly cleared from the plasma and is excreted largely unchanged in the urine, although small amounts of the glycine and taurine conjugates have been detected. Pharmacokinetic

studies have revealed that the plasma clearance of 2,4-D is saturable. The chemical is distributed widely among the tissues of the body; the highest concentrations being found in parenchymal and excretory organs. Brain 2,4-D concentrations are generally low but may increase if 2,4-D is administered at intoxicating doses. These conclusions agree with those of others (Gehring and Betso, 1978, Leng, 1977; Mullison, 1986; Veterans Administration, 1981a and b; World Health Organization, 1984).

4.2. Human studies

The percutaneous absorption of [^{14}C]2,4-D (purity not stated) and the urinary excretion of [^{14}C]2,4-D after dermal or intravenous administration was studied in male subjects (age and weight not stated) (Feldman and Maibach, 1974). After intravenous administration of a tracer (1 uCi) dose of [^{14}C]2,4-D, $100 \pm 2.5\%$ of the dose was excreted in the urine in 5 days; the half-life for excretion was 13 h. After dermal administration of [^{14}C]2,4-D (1 uCi; 4 ug/sq. cm.), $5.8 \pm 2.4\%$ of the dose was excreted in the urine.

The pharmacokinetics of 2,4-D was studied in five male subjects aged 29 to 40 years and weighing 70 to 90 kg (Sauerhoff *et al.*, 1977). Each subject ingested 5 mg/kg of analytical grade 2,4-D either as a slurry in milk or as the powder followed by water. Blood samples were collected at 1, 4, 8, 12, 24, 36, 48, 72, 96, 120, and 144 h and urine samples at 12-h intervals after dosing. Average $t_{1/2}$ for absorption of 2,4-D was 3.8 h (range 1.67 to 4.20 h), $t_{1/2}$ for clearance from plasma averaged 11.6 h and $t_{1/2}$ for the urinary elimination averaged 17.1 h (range 10.2 to 28.4 h). Most (82%) of the 2,4-D was excreted unchanged, but 13% was excreted as conjugates. The clearance of 2,4-D from the plasma and the urinary elimination of 2,4-D followed first-order kinetics, which could be described by a one-compartment model although one subject showed biphasic clearance of 2,4-D. The authors concluded that 2,4-D was rapidly absorbed from the intestinal tract and rapidly excreted in the urine and would not be expected to accumulate in the body after repeated oral dosing at usual levels of exposure.

Plasma and urine 2,4-D concentrations have been measured in occupationally exposed workers (Kolmodin-Hedman and Erne, 1980). Four male subjects (mean age of 39 years) involved in spraying an emulsion containing 2% 2,4-D (neither purity nor amine or ester content was stated) and kerosene were studied. Blood samples were collected before the start of spraying, immediately after spraying, and at the end of the exposure week; urine samples were

collected at the start and at the end of the days of exposure or, in another study, at 12-h intervals during the exposure period. Concentrations of 2,4-D in air ranged from 0.1 to 0.2 mg/m³. Plasma 2,4-D concentrations ranged from 0.02 to 0.2 ug/ml and varied considerably because of intermittent exposure; after exposure, plasma 2,4-D concentrations dropped overnight to near the detection limit (0.02 ug/ml). Urine 2,4-D concentrations ranged from 3 to 14 ug/ml and the mean peak was 8 ug/ml. Mean 24-h excretion of 2,4-D amounted to 9 mg. The authors concluded that 2,4-D may enter the body by inhalation or dermal absorption and that urinary elimination of 2,4-D is rapid.

Taskar et al. (1982a and b) measured serum and urine 2,4-D concentrations in 11 male subjects aged 19 to 31 years (weights not stated), who applied 2,4-D (DMA-4, Dow Chemical Co.). Body surface exposure was estimated by measuring the 2,4-D content of gauze pads (620 cm²: 0.67 ft²) attached to the chest and back of each subject and of a paper cap (406 cm²: 0.44 ft²) worn by the subjects. Blood samples were collected before after exposure. Urine was collected before and after exposure, during the day of exposure (3 h after spraying) and twice a day for four to seven days after exposure. At the end of the exposure period, 2,4-D residues of 564, 532, and 319 ug/m² were found on the head, chest, and back, respectively, of the subjects. Serum 2,4-D concentrations (expressed as "amount of 2,4-D measured as phenolics in serum") at the end of exposure averaged 167 ng/ml (range 15.6 to 484.2 ng/ml). The serum 2,4-D concentrations measured 1, 2, and 3 days after exposure varied considerably. In some subjects a progressive decline in serum 2,4-D concentrations was noted, but in other subjects the 2,4-D content of samples taken on the second day after exposure was greater than that in samples taken on the first or third day after exposure. In some subjects, serum 2,4-D concentration was highest on the third day after exposure. Urine 2,4-D levels were apparently measured for 84 h after exposure, but no units of concentration were reported in the paper; hence the urine data cannot be evaluated. The authors asserted that serum residues correlated positively with the 2,4-D concentration in the air and with the amount of 2,4-D deposited on the head and back of the subjects. However, no air 2,4-D concentrations were reported in the paper and the results of statistical analyses were not presented.

Absorption and urinary excretion of 2,4-D was studied in 36 applicators (sex, age, and weight not stated) occupationally exposed to a 2,4-D/picloram (4-amino-3,5,6-trichloropicolinic acid) mixture (Dow Chemical Tordon 101) or a 2,4-D/dichloroprop (2-(2,4-dichlorophenoxy)propanoic acid) mixture (Pfizer

Chemical) (Libich et al., 1984). In one study conducted in 1979, urine 2,4-D concentrations averaged 6.17 ± 7.69 (range 0.27 to 32.74) mg/kg (L) in one group of workers and 3.16 ± 2.85 (range 0.63 to 12.35) mg/kg (L) of urine in another group of workers. In a second study conducted in 1980, improved working procedures were introduced and workers were instructed on the procedures that were required; moreover, air 2,4-D concentrations were also measured. In three groups of workers, urine 2,4-D concentrations averaged 1.42 ± 1.76 (range = 0.04 to 8.15), 1.72 ± 1.50 (range = 0.15 to 5.45), and 2.55 ± 1.72 (range = 0.44 to 5.07) mg/kg (L); respective air 2,4-D concentrations were 7.1 ± 4.9 (1.0 to 19.5), 13.5 ± 7.6 (0.4 to 35.3), and 55.2 ± 30.7 (16.2 to 91.3) $\mu\text{g}/\text{m}^2$. Finally, a model was devised that related urine 2,4-D concentrations to daily exposure levels of airborne 2,4-D and allowed the use of urine 2,4-D concentrations as exposure guides.

The urinary elimination of 2,4-D after direct and indirect exposure of forestry workers to 2,4-D has been studied (Frank et al., 1985). Seven subjects (5 males and 2 females) weighing 51 to 84 kg and aged 20 to 38 years who applied formulations containing 2,4-D isooctyl esters (Estron LV-600 and Pfizer 2,4-D Ester LV600) were used. In an experiment conducted in 1981, three workers were directly exposed to 2,4-D for 65 h during an 11 day period; for the three workers, the highest daily excretion of 2,4-D in the urine was 0.30, 0.94, and 9.59 $\mu\text{g}/\text{kg}$ body weight. In an experiment conducted in 1982, three workers were exposed to 2,4-D for 50, 51, and 39 h during an 18 day period; the highest daily excretion of 2,4-D in the urine for each of the workers was 7.73, 8.37 and 22.2 $\mu\text{g}/\text{kg}$ body weight/day. For one subject who was sprayed directly, it was estimated that 0.44% of the applied dose was absorbed, and the highest daily excretion of 2,4-D in the urine was 4.75 $\mu\text{g}/\text{kg}$ body weight/day. Urinary excretion of 2,4-D was followed for 8 days in this subject; a half-residue decline of 16 h determined. Further studies showed that 2,4-D persisted in urine samples collected during the post-spray period. Analysis of surface swabs of vehicles, helicopters, and living quarters revealed contamination of all internal surfaces with 2,4-D which were probably the source of the 2,4-D detected in urine samples collected in the post-spray period. The authors calculated that, assuming a half-life of 18 h for 2,4-D, in the worker excreting the most 2,4-D (22.2 $\mu\text{g}/\text{kg}$ body weight/day), the maximum dose of 2,4-D absorbed amounted to 60 $\mu\text{g}/\text{kg}$ body weight/day.

Dermal absorption and urinary excretion of 2,4-D were studied in four subjects (ages 24 to 57 yr, sex and weight not stated) exposed under field

conditions to 2,4-D amine salts (the commercial formulations used were not described) (Grover *et al.*, 1986). Urine samples were collected before, during and for 4 to 7 days after the spray operations; dermal exposure was estimated by measuring the 2,4-D content of patches fastened both outside and underneath the clothing and by measuring the amount of 2,4-D removed by washing the hands with a sodium bicarbonate solution after the spray exposure. In all subjects, the urinary excretion of 2,4-D increased after each spraying operation. The amount of 2,4-D excreted was a function of the number of consecutive exposures, the number of days between exposures, and the time elapsed since the last exposure. The cumulative total amounts of 2,4-D excreted were a function of the number of consecutive exposures, the number of days between exposures and the time elapsed since the last exposure. The cumulative total amounts of 2,4-D excreted ranged from 215 to 6,258 ug. Statistical analysis revealed a positive correlation between the amount of 2,4-D applied and the cumulative amount of 2,4-D excreted. In addition, there was a positive and significant correlation between the amount of 2,4-D deposited on the hands and the amount of 2,4-D excreted in the urine.

4.3. Evaluation of pharmacokinetics and metabolism

Studies on the physiological disposition of 2,4-D in human subjects show that 2,4-D is absorbed after oral or dermal administration and is rapidly and almost completely eliminated from the body by urinary excretion. The half-life for clearance of 2,4-D from the body is <24 h. After occupational exposure to 2,4-D, dermal absorption appears to be the major route of entry into the body. These conclusions agree with those of others (IARC, 1987; Veterans administration, 1981a and b; World Health Organization, 1984).

The relevant pharmacokinetic studies provide evidence of a reliable and constant relationship between exposure, uptake and urinary elimination of 2,4-D in workers exposed over several days and who have achieved steady state pharmacokinetics. These studies also indicate that, in workers who use 2,4-D regularly, the amount excreted in the urine over a 24-hour period is an reliable measure of the absorbed systemic dose.

5. EXPOSURE ASSESSMENT

5.1. Occupational Exposure to 2,4-D.

Frank *et al.* (1985) reported a study on exposure to 2,4-D, by measurement of urinary excretion for 2 sets of workers. Crew 1, which

included 2 mixer-loaders and a supervisor, wore full protective gear (including respirator) and worked over an 11-day period. Approximately 2,200 ha were sprayed at an application of 2 kg/ha. Crew 2, included 1 mixer-loader, 1 mixer-balloon (flagger) man, and 1 balloon man, also wore protective gear but did not consistently wear respirators. The spray period was 18 days. Approximately 4,000 ha were sprayed with 1.3 kg/ha. Highest daily excretion for crew 1 ranged from 0.30 to 9.59 ug/kg body weight while that for crew 2 was considerably higher, ranging from 7.73 to 22.2 ug/kg body weight. Total excreted doses ranged from 136 ug (supervisor) to 1,600 ug (mixer-loader).

In a study by Lavy et al. (1982), urinary excretion data were utilized to estimate total absorbed dose of 2,4-D for 3 crews involved in aerial application of the herbicide to 40 ha tracts of forest in Washington State. The herbicide was applied at the rate of 2.1 kg/ha. Crews were involved in two applications each: one in which conventional clothing was worn (no protective gear), the other in which protective gear was worn (without respirator). Monitoring was performed in such a way as to allow estimates of exposure resulting from dermal and inhalation routes, results of which indicated that exposure was primarily due to dermal contact. This and a later study (Lavy et al., 1987) were not considered further because conditions were not typical of Ontario.

Libich (1981) and Libich et al. (1984) reported studies to assess exposure to those who spray roadsides, power lines and rights-of-way for Ontario Hydro. Spraying was done by backpack (handheld gun or mist blower) or from vehicle (truck or all-terrain vehicle, using handheld gun), and mixtures of either 2,4-D and 2,4-dichloropropanoic acid (2,4-DP) (1:1) or 2,4-D and picloram (4:1) were used. Little information was given concerning the amounts of herbicide used, but a recent survey (DHS, 1987) indicates that Ontario Hydro applies 2,4-D/2,4-DP mixture at rates up to 5.5 kg 2,4-D/ha and 2,4-D/picloram mixture at a rates up to 22.7 kg 2,4-D/ha. Information on use of protective clothing was also limited to statements that there was general use of gloves, that clean coveralls were issued daily and that wash-up facilities were provided. Average daily intake, approximated by average excretion on Thursday of each spray week, was 3.53 mg, 3.45 mg, and 4.86 mg for rights-of-way sprayers using handheld gun, roadside sprayers using handheld gun, and rights-of-way sprayers using mist blowers, respectively.

Occupational exposure to 2,4-D was assessed for commercial lawn care

specialists who apply 2,4-D in combination with other herbicides including MCPP [2-(2-methyl-4-chlorophenoxy)propanoic acid] and dicamba (Yeary, 1986). The diluted mixture contained 2,4-D, MCPP and dicamba in a ratio of 12:6:1. Personnel had been involved in application of herbicides for 3 weeks prior to this analysis and, therefore, were assumed to exhibit steady-state body burdens of 2,4-D. Exposure was estimated by analysis of urinary excretion with the assumption that, at steady-state, the 24-hour urinary excretion of 2,4-D was a reasonable estimate of daily absorbed dose. While mixing herbicide, employees wore protective clothing including gloves, apron or coveralls, face protection, and rubber boots. However, during actual spraying, wearing of gloves and eye protection was optional while rubber boots and clean uniforms (long pants, short-sleeve shirt) were considered standard apparel. Solutions were applied at a rate of 16 L per 93 m² (1.3 kg 2,4-D/ha), and 3,000 to 4,500 L were applied daily (adequate treatment for 2 to 3 ha). Personnel from five locations were monitored; however, in one of these locations spray activities were interrupted by rain. For the other four spray areas, mean urinary excretion values were reported to be 1.4, 3.2, 6.3, and 2.5 ug/kg body weight/day. Average exposure for the four groups combined was 3.3 ug/kg/day.

Grover et al. (1986) assessed exposure in farmers, exposed multiple times in one season to 2,4-D under field conditions in Saskatchewan. Farmers used their own tractors and ground rigs and applied 2,4-D at rates applicable to crop and control needs. Dermal exposure was estimated by use of patches and handwashes and total exposure by urinary excretion values. Subjects wore garments consisting of two layers of cotton but did not use respirators. Number of exposures per subject ranged from 1 to 7, with those having more exposures excreting greater amounts of 2,4-D in terms of kilogram of herbicide applied. Application rates ranged from 0.35 kg/ha to 0.63 kg/ha, averaging 0.43 kg/ha. Cumulative urinary excretion ranged from 0.2 mg to 6.3 mg 2,4-D, with an average value of 1.6 mg excreted. One subject who was monitored for only one exposure excreted a total of 0.3 mg as a result of spraying 71 ha at a rate of 0.35 kg/ha.

5.2. Estimation of Exposure

Data on urinary excretion of 2,4-D from each of the above reports were used to estimate absorbed dose under various work conditions most typical of the Ontario use pattern of 2,4-D. These estimates are contained in Table 2.

For each of the above studies, an average daily dose was estimated. Information on days per year exposed and number of years exposed (Table 2) were derived from data in a recent survey (DHS, 1987). If such data were not available, values for these parameters were assumed using best professional judgement.

Table 2. Occupational exposure of persons involved in the application of 2,4-D by various methods

Method of Application	Occupation	Average daily intake (mg/person)	Days/ year Exposed	Years Exposed	Total Intake (mg)
Frank <u>et al.</u>, 1985					
Helicopter	mixer-loader	1.04	12	20	250
	mixer-flagger	0.46	12	20	111
	flagger	0.34	12	20	80
Airplane	mixer-loader	0.15	12	20	36
	supervisor	0.005	12	20	1
Libich, 1981					
Packsprayers					
handheld gun	rights-of-way sprayer	3.5	60	10	2107
	roadside sprayer	3.4	60	10	2070
Mist blower	rights-of-way sprayer	4.9	60	10	2895
Yeary, 1986					
Hand and tank	commercial sprayer	0.29	66	13	245
Grover <u>et al.</u>, 1986					
Tractor	farmer	0.48	14	25	166

In the study by Frank et al. (1985), total urinary excretion data were reported (including post-spray excretion) and average daily dose was approximated by dividing the total urinary excretion by the number of reported spray days. In contrast, the study by Libich (1981) reported urinary excretion on (often non-consecutive) spray days, but not on post-spray days. Libich (1981) indicated that, for successive daily exposure, urinary excretion after the fourth day should approximate the daily exposure. Also, the reported measurements were made generally during weeks in which spraying activities were conducted Monday through Friday (personal communication from Libich). Therefore, average daily urinary excretion reported on Thursdays and Fridays was assumed to approximate average daily intake. Information on exposure to farmers spraying 2,4-D (Grover et al., 1986) was expressed as total urinary

excretion of 2,4-D for the total number of spray operations (all occurring within a short period of time). Average daily intake was approximated by dividing total urinary excretion per farmer by the number of spray operations performed by that farmer. The study of Yeary (1986) reported 24-hour urinary excretion of 2,4-D for five groups of commercial herbicide sprayers, in units of mg 2,4-D/kg body weight. The sprayers had been working almost daily for three weeks, so that this 24-hour sample was used as an approximation of average daily intake. Each study reported the average percent of 2,4-D recovered by the chromatographic method utilized, and reported amounts of 2,4-D excreted were corrected for less than complete recovery. It was assumed that the studies utilized reported relatively normal spray practices, i.e. that parameters such as rate of 2,4-D applied and use of protective clothing fell within normal limits. If body weight and total urinary output were not supplied, values of 70 kg and 1.4 L/day were utilized where needed.

5.3. Evaluation of Exposure

It is clear that exposure to 2,4-D is dependent on the rate of application of the herbicide. Rights-of-way sprayers use 2,4-D at the highest rate of application and their daily exposure was higher than other groups. Hydro-line workers may be exposed to the 2,4-D in amounts from 0.005 - 5 mg/person/spraying day. Exposure in this setting is highly variable and depends upon the nature of the work performed (mixer-loader, sprayer, flagger, etc.) and the extent to which precautionary procedures are followed and protective gear is worn. Commercial lawn applicators were found to receive a daily dose of approximately 0.3 mg/person/spraying day while farmers were estimated to receive about 0.5 mg/person/spraying day. Variation between these groups is due to differences in spray equipment, terrain and to the degree to which the operators come into contact with concentrated formulations. The latter is the most probable reason for the low levels of exposure in commercial lawn applicators who were reported to take appropriate precautions when mixing the formulation. Lifetime exposure to 2,4-D is related to the number of days of spraying and the number of years in the occupation.

6. GENOTOXICITY

It is widely accepted that tests of genetic activity, conducted using appropriate in vitro and in vivo systems, may indicate possible carcinogenic activity. Positive results in genetic toxicity tests; however, cannot be said to unequivocally predict carcinogenicity since these tests only measure a limited number of events putatively associated with the carcinogenic process. Similarly, negative results in tests of genotoxicity cannot be considered to provide conclusive evidence against carcinogenicity since agents may act to produce cancer through processes not detected by the currently available short-term tests.

Positive results for genotoxic activity obtained in in vitro systems such as prokaryotes, fungi and cultured mammalian cells may suggest that similar effects could occur in animals in vivo. It is therefore important to assess the genetic effects of chemicals in vivo to observe if similar effects are observed in animals exposed under appropriate doses and routes of administration. Positive results in in vivo test systems provide important additional evidence that the chemical is genotoxic and are regarded as being of greater relevance than effects in lower order organisms (IARC, 1987).

Attempts to relate genotoxic effects to carcinogenicity must take into consideration possible mechanisms of action and evidence which describes the relationship between the test system and carcinogenicity in whole animals or humans. In this regard it is important to note that correlations between genotoxicity and carcinogenicity are far from perfect and, indeed, are considered by some investigators (Mendelsohn, 1985) to be of limited value for regulatory purposes. It is therefore essential that the interpretation and regulatory use of the results of short-term genotoxicity tests encompass a critical scientific evaluation of the data, keeping in mind its possible relevance to human exposure conditions.

The genotoxicity of 2,4-D has been studied extensively in a wide variety of in vitro and in vivo test systems. As 2,4-D is a highly active and toxic herbicide, it is inappropriate to include those genotoxicity studies conducted on plants and plant tissues. The pivotal studies concerning the potential genotoxicity of 2,4-D are discussed below.

6.1. In Vitro Studies

The salient in vitro genotoxicity studies on 2,4-D are summarized in Table 3. Mutagenicity of 2,4-D has been tested repeatedly in several strains of

Salmonella typhimurium (Klopman et al., 1985; Zetterburg et al., 1977; Anderson et al., 1972; Shiraus et al., 1978; Mortelmans et al., 1984; Rashid and Mumma, 1983). Concentrations of up to 10 mg/plate, a level which produced toxicity in several strains of Salmonella, failed to induce an increase in mutation frequency. In an assay measuring differential toxicity of 2,4-D to Bacillus subtilis, Waters et al. (1982) noted positive results, suggesting that 2,4-D may inhibit DNA repair in this test system. However, no effect on DNA repair, as measured by differential toxicity, was noted in Escherichia coli polA⁻ strain (Waters et al., 1982) and Shirasu et al. (1976) failed to obtain positive results in the B. subtilis assay. Ahmed et al. (1977a) reported that a formulation of "2,4-D-Fluid" (purity and composition not given) induced unscheduled DNA synthesis (UDS) in cultured human fibroblasts; however, Probst et al. (1981) found no increase in UDS in primary rat hepatocyte cultures and Simmon (1979) found no effect of 2,4-D on UDS in human embryonic lung cells at concentrations up to 100 mg/L.

Table 3. In vitro studies on 2,4-D.

<u>Test Organism</u>	<u>Result</u>	<u>Reference and comments</u>
<u>Salmonella typhimurium</u> histidine reversion assay	-	Klopman <u>et al.</u> (1985). Neither the purity nor the concentration of the 2,4-D was given in the paper.
<u>Salmonella typhimurium</u> histidine reversion assay	-	Zetterburg <u>et al.</u> (1977). No effects at pH values of 4.3 or 6.8.
<u>Salmonella typhimurium</u> histidine reversion assay	-	Anderson <u>et al.</u> (1972). pH not stated but is probably that of the medium. Spot test system with concentration of 50 ug/plate.
<u>Salmonella typhimurium</u> reversion assay	-	Shirasu <u>et al.</u> (1976).
<u>Salmonella typhimurium</u> reversion assay in TA98, TA100, TA1535 and TA1567 strains.	-	Mortelmans <u>et al.</u> (1984). Used five concentrations over 2 orders of magnitude of 2,4-D acid, n-Butyl, isooctyl esters and amine with and without activation by rat S9 microsomes
<u>Salmonella typhimurium</u> reversion assay in G46, TA98, TA1000, TA1535, TA1537, TA1538, D3052 and C3076 strains.	-	Probst <u>et al.</u> (1981). Used 10000-fold concentration gradient replicated 4 times of 2,4-D acid with and without activation by Arochlor 1254 induced rat S9 microsomes
<u>Salmonella typhimurium</u> reversion assay in TA97, TA98, TA100, TA1535 and TA1538 strains.	-	Rashid and Mumma (1983). No effects with the alanine, aspartic acid, leucine, methionine and tryptophan conjugates of 2,4-D at concentrations of 10, 100 and 1000 ug/plate
<u>Salmonella typhimurium</u> reversion assay in TA97, TA98, TA100, TA1535 TA1537 and TA 1538 strains.	-	Moriya <u>et al.</u> (1983). Dose response was determined up to a concentration of 5000 ug/plate.

<u>Escherichia coli</u> DNA repair assay.	-	Waters <u>et al.</u> (1982).
<u>Escherichia coli</u> WP 2 tryp. reverse mutation assay.	-	Nagy <u>et al.</u> (1975). Spot test with undefined concentrations of 2,4-D.
<u>Escherichia coli</u> reversion assay	-	Shirasu <u>et al.</u> (1976).
<u>Escherichia coli</u> WP 2 tryp. reverse mutation assay.	-	Moriya <u>et al.</u> (1983). Dose response was determined up to a concentration of 5000 ug/plate.
<u>Bacillus subtilis</u> DNA repair assay.	+	Waters <u>et al.</u> (1982).
<u>Bacillus subtilis</u> recombination assay.	-	Shirasu <u>et al.</u> (1976).
<u>Saccharomyces cerevisiae</u> mitotic recombination assay.	+-	Zetterburg <u>et al.</u> (1977). Effects only observed at pH $\leq$ 4.5, possibly because of lack of uptake of dissociated form of molecule.
<u>Saccharomyces cerevisiae</u> mitotic recombination assay.	-	Klopman <u>et al.</u> (1985). Comments as for above.
<u>Saccharomyces cerevisiae</u> host mediated mitotic recombination assay.	-	Zetterburg <u>et al.</u> (1977).
<u>Saccharomyces cerevisiae</u> RAD 18 strain reverse mutation assay.	+-	Zetterburg (1978). Again, effects only observed at pH $\leq$ 4.5, possibly because of lack of uptake of dissociated form of molecule.
Mitotic gene conversion assay in <u>Saccharomyces cerevisiae</u> D4 strain.	+	Seibert and Lemperle (1974). 2,4-D increased convertants 5 to 6-fold at pH 4.5. (See comments on Zetterburg <u>et al.</u> , 1977 above)
Unscheduled DNA synthesis in human fibroblast cells.	+	Ahmed <u>et al.</u> (1977a). Assay with and without rat S9 microsomal activation was positive at all concentrations tested. The 2,4-D formulation used was not clearly identified except as 2,4-D fluid. The structure was shown to be that of the acid but it was stated that the material was water soluble, suggesting that the amine or the salt may have been used. The possible involvement of mutagenic contaminants cannot be ruled out.
Unscheduled DNA synthesis in human embryonic lung cells.	-	Simmon (1979). Cells of the WI-38 strain exposed to concentrations ranging from 0.1 to 100 mg/L in presence and absence of microsomal activation
Unscheduled DNA synthesis in rat hepatocyte cells.	-	Probst <u>et al.</u> (1981). Hepatocytes from Fischer 344 rats did not show increased UDS at concentrations up to 1,000 mg/L.
Forward mutation in Chinese hamster V79 cells.	+	Ahmed <u>et al.</u> (1977b). A 12-fold increase in forward mutation at a concentration of 4 mg/L at which 60% of cells were killed. The 2,4-D formulation used was not clearly identified except as 2,4-D fluid. The structure was shown to be that of the acid but it was stated that the material was water soluble, suggesting that the amine or the salt may have been used. The possible involvement of mutagenic contaminants cannot be ruled out.

Thymidine kinase gene mutation assay in mouse lymphoma cells (L5178Y).	-	Klopman et al. (1985). Neither the purity nor the concentration of the 2,4-D was given in the paper.
SCE in human lymphocytes.	+	Korte and Jalal (1982). Increases in SCEs at concentrations of 2,4-D $\geq$ 10 mg/L in <u>in vitro</u> system.
SCE in human lymphocytes.	+ -	Turkula and Jalal. (1985). Statistically significant increase only at 50 mg/L, none at 100 and 250 mg/L. No dose response was observed.
Mitotic index in fetal bovine kidney cells.	+ -	Bongso and Basrur (1973). Increase in mitotic index up to 10 mg/L with a decrease at concentrations up to 1000 mg/L. Some indication of interference with spindle formation. Formulation of 2,4-D not specified.
Mitotic index in bovine peripheral blood cells.	+ -	Bongso and Basrur (1973). Increase in mitotic index up to 10 mg/L with a decrease at concentrations up to 1000 mg/L. No other indications of interference cell division. Formulation of 2,4-D not specified.
Chinese hamster ovary cells, SCE.	-	Linnainmaa (1984). Both pure (Acid) and commercial formulations of 2,4-D and MCPA (amine of 2,4-D and isooctyl ester of MCPA) at non-toxic concentrations of 10^{-3} , 10^{-4} and 10^{-5} M caused some increases in the presence or absence of activation by rat liver S9 fraction. Although these increases were, in some cases, statistically significant, they did not show a dose response and, in most cases, resulted from somewhat lower rates in the control groups. The author concluded that neither of these compounds appeared to act directly on DNA.

In tests employing yeasts, Zetterburg et al. (1977) noted a dose-dependent increase in the frequency of mitotic gene conversion and mitotic recombination in Saccharomyces cerevisiae; however, this effect was found only at pH 4.5 and 4.3 and not at higher pH values. At lower pHs survival was markedly affected. Zetterburg (1978) concluded that the pH dependency on mutation frequency was due to the fact that, at lower pHs, 2,4-D is in an molecular form which is readily taken up cells in culture. This conclusion is supported by Zetterburg's observation that 2,4-D did not produce mutagenic affects in S. cerevisiae when tested in a host-mediated assay using mice.

Studies by Korte and Jalal (1982) and Turkula and Jalal (1985) were said by these authors to demonstrate that 2,4-D produced sister chromatid exchanges (SCEs) in cultured human lymphocytes. Effects noted in these studies were, in most cases, marginal, occurred at near-toxic doses and failed to meet accepted criteria for positive results (Waters et al., 1982). In addition, Waters et al., (1982) in a definitive series of tests, did not observe any effect of 2,4-D on SCE frequency in CHO cells while Linnainmaa (1984) observed only a

marginal effect of 2,4-D on SCE frequency in CHO cells which failed to meet accepted criteria for positive results.

In conclusion, it may be stated that there is no firm evidence that 2,4-D induces SCE's in cultured mammalian cells. This view is substantiated by the results of in vivo tests in animals and man.

6.2. In Vivo Studies

6.2.1. Animal Studies

Studies on the genotoxicity of 2,4-D conducted in animals are summarized in Table 4. As pointed out above the marginally positive results for SCE reported in in vitro systems have not been noted in whole animal studies. Linnainmaa (1984) did not find any increase in SCEs in circulating lymphocytes of rats treated for one week with 2,4-D at a daily dose (close to toxic) of 100 or 200 mg/kg. Similarly, Linnainmaa (1984) failed to demonstrate an increased frequency of SCEs in the bone marrow of hamsters treated with 100 mg/kg/day of 2,4-D for 7 days. Lamb et al. (1981) likewise noted no increase in the frequency of SCEs in the bone marrow of mice given 2,4-D. Pilinskaya (1974); however, reported a slight effect of 2,4-D on chromosomal aberrations in the bone marrow of mice treated at intoxicating doses of 100 or 300 mg/kg but no effects were noted at 10 or 50 mg/kg. A mouse micronucleus test conducted at an i.p. dose of 100 mg/kg produced negative results (Jenssen and Renberg, 1976). A dominant lethal assay in ICR Ha Swiss mice given 125 mg/kg 2,4-D was likewise negative (Epstein et al., 1972).

Table 4. Mutagenicity of 2,4-D in animal systems.

<u>Test Organism</u>	<u>Result</u>	<u>Reference and comments</u>
Male Wistar rats, peripheral lymphocyte SCE.	-	Linnainmaa (1984) Pure (acid) 2,4-D and MCPA did not cause increases in peripheral lymphocyte SCE when fed for one week at doses of 100 and 200 mg/kg body weight/day by gavage.
Chinese hamster bone marrow cell SCE.	-	Linnainmaa (1984) No elevations were observed in the case of 2,4-D when both were dosed by gavage at 100 mg/kg body weight/day for 7 days.
Mouse testicular DNA synthesis.	+	Seiler (1978). A 29% reduction in thymidine uptake after oral dosing of mice with 200 mg/kg. The dose is near to the toxic and results may be general toxic response. No dose-response measured.
C57BL/6N mouse bone marrow SCE.	-	Lamb <u>et al.</u> (1981) failed to demonstrate significant elevations in mice fed diets resulting in daily doses of 40 and 20 mg each of 2,4-D, 2,4,5-T (containing TCDD) /kg body weight/day for 8 weeks.

Mouse bone marrow cells, chromosomal aberrations.	+	Pilinskaya (1974). Only observed at doses of 100 and 300 mg/kg body weight in combination with intoxication. Not observed at 10 and 50 mg/kg body weight.
Mouse micronucleus test.	-	Jenssen and Renberg (1976). No effects observed at an IP dose of 100 mg/kg body weight.
Nondisjunction assay in <u>Drosophila melanogaster</u>	-	Magnusson <i>et al.</i> (1977). No effect at 100 ppm 2,4-D in diet.
Chromosome loss assay in <u>Drosophila melanogaster</u>	-	Magnusson <i>et al.</i> (1977). No effect at 100 ppm 2,4-D in diet.
Recessive lethal assay with Karsnas and Muller 5 strains of <u>Drosophila melanogaster</u>	+	Magnusson <i>et al.</i> (1977). Effect with 2,4-D statistically significant at 1000 ppm in diet.
Recessive lethal assay with Berlin K strain of <u>Drosophila melanogaster</u>	-	Vogel and Chandler (1974). No effects at dietary concentrations of 500 and 1000 ppm.
Genetic mutations in stable and unstable strain of <u>Drosophila melanogaster</u>	+-	Rasmuson and Svahlin (1978). Positive results in the unstable strain, negative in the stable strain.
Dominant lethal assay in ICR Ha Swiss mice.	-	Epstein <i>et al.</i> (1972). Single injection (IP) of 2,4-D at 125 mg/kg body weight.

There is some evidence from studies in Drosophila melanogaster that 2,4-D may produce mutations; however, the evidence in this regard is conflicting. Magnusson *et al.* (1977) noted negative results in a nondisjunction assay and a chromosome loss assay in Drosophila given a diet containing 100 ppm 2,4-D, but noted a statistical increase in a recessive lethal assay in Drosophila given a diet containing 1000 ppm 2,4-D. Magnusson *et al.* (1977) concluded that the effect was weak, amounting to only 2-3 times the control levels. Rasmuson and Svahlin (1978) reported an enhanced somatic mutation frequency in unstable but not in stable strains of Drosophila; however, the response reported was weak. Compared to the classical mutagen ethyl methane sulphonate, which induced a 3.28% increase in mutation frequency, 2,4-D induced only a 0.69% increase. The control mutation frequency was 0.075%. Other studies in Drosophila (Vogel and Chandler, 1974) have failed to demonstrate any significant effect of 2,4-D on the frequency of recessive lethal mutations.

The available animal studies do not provide convincing evidence that 2,4-D is genotoxic. Marginally positive results, when reported, have occurred at near-toxic concentrations. The relevance of these positive findings to risk assessment is questionable, particularly in view of the fact that most studies have produced negative results.

6.2.2. Human Studies

There is no evidence that, under conditions of manufacture or use, 2,4-D produces genotoxic effects in humans. In a review on 2,4-D, WHO (1984) reported five studies on workers exposed to 2,4-D under manufacturing or use situations. In no case were any chromosomal abnormalities noted.

Linnainmaa (1983a and b and 1982) compared the incidence of lymphocyte SCEs in herbicide workers with that in controls and found no exposure related effects. Comparison of 2,4-D levels in the urine of exposed workers and frequency of SCEs failed to show a dose response. In both the control and exposed group, SCEs were significantly elevated in those workers who smoked although no differences between control and exposed were observed in the degree of elevation. Elevation of SCEs in smokers would be expected and, as such, is a positive control for the study. The results failed to demonstrate synergism between 2,4-D exposure and smoking.

Mustonen et al. (1986) reported no difference between frequency of chromosomal aberrations in lymphocytes from workers exposed to 2,4-D and control workers. Exposure was confirmed by urinary analysis. As reported above, the frequency of aberrations was higher in smoking than non-smoking workers in both exposed and control groups.

Several older studies of chromosome aberrations and SCEs in humans exposed to 2,4-D were reviewed recently by IARC (1987). The results of these studies have been uniformly negative.

6.3. Evaluation of Genotoxicity

There is no evidence that 2,4-D is mutagenic in the Ames Salmonella test or in Escherichia coli; however, some conflicting data have been reported in other bacterial systems. Both positive and negative results have been noted in differential toxicity studies in B. subtilis. The significance of these positive results is questionable in view of the fact that this test correlates poorly, if at all, with carcinogenicity. A formulation of 2,4-D "fluid" of unspecified composition has been reported to induce unscheduled DNA synthesis in human fibroblasts; however, other studies with pure formulations of 2,4-D have been unable to confirm these findings in tests involving human embryonic lung cells and cultured rat hepatocytes. It is possible that the positive results reported in human fibroblasts with 2,4-D "fluid" may represent a true finding; however, the possibility that the effects noted were due to impurities or other anomalies

in the test cannot be discounted. They also reported that 2,4-D induced mutations in yeast but positive effects were noted only at a pH below 4.5, leading the authors of these studies to conclude that effects were dependent entirely on pH of the culture media.

There are studies indicating that 2,4-D produces SCEs in cultured human lymphocytes but not in hamster embryo cells. The significance of these findings is questionable in light of the fact that several in vivo studies involving rats, mice, hamsters and humans have not shown any effects on SCEs in lymphocytes or bone marrow cells when 2,4-D was administered by appropriate routes at up to toxic doses. In addition, SCEs in vitro cannot be viewed as reliable predictors of carcinogenicity (Brusick et al., 1983). There is one report that 2,4-D induced chromosomal aberrations in mouse bone marrow cells (Pilinskaya, 1974); however, the significance of this finding is questionable because the animals were given a dose corresponding to the LD₅₀.

Conflicting data exist on the mutagenic activity of 2,4-D in Drosophila. Unstable strains appear to show weakly positive effects while more stable strains appear to be resistant even at very high dietary concentrations (e.g. 1,000 ppm). A mouse micronucleus test and a dominant lethal assay in mice conducted at doses of 100-125 mg/kg produced negative results.

In vitro studies on the genotoxicity of 2,4-D, in some cases, produced conflicting results; however, there is no convincing evidence that 2,4-D produces mutagenic effects when it is tested in in vivo systems. Overall, the pattern of responses observed in both in vitro and in vivo tests indicates that 2,4-D is not genotoxic.

7. PATHOLOGY AND CARCINOGENICITY

The potential carcinogenicity of chemicals is determined primarily by epidemiological studies in humans or by long-term animal experiments. This section deals with studies in experimental animals chronically exposed to 2,4-D. Several animal experiments have been conducted using mice and rats, but most of these were completed over a decade ago and do not meet current standards for determining carcinogenicity (Innes et al., 1969; Hansen et al., 1971; Arkipov & Koxlova, 1974). Rueber (1983) also published an interpretation of the Innes et al. and Hansen et al. studies (1983). Two working groups of the International Agency for Research on Cancer have reviewed all of this data and considered them inadequate for an assessment of carcinogenicity (IARC, 1977, 1982). These studies have not been reviewed in detail for this report

Animal experiments in rats and mice have recently been conducted for the Industry Task Force on 2,4-D Research Data at Hazleton Laboratories America, Inc. in Vienna, Virginia. These studies were completed in 1986 and were conducted according to current good laboratory practices (GLP) requirements. The final study reports and selected rat brain sections, including all brain gliomas, were examined and form the primary basis for the assessment in this section.

7.1. Industry Task Force 2,4-D Rat Study

Groups of 60 male and 60 female weanling Fischer 344 rats were treated with 2,4-D (Acid, 97.5% pure) in the diet at levels of 0, 1, 5, 15 or 45 mg/kg body weight/day for up to 104 weeks (Hazleton, 1986). At 52 weeks, 10 animals in each group were sacrificed and subjected to pathological examination. The doses were selected based upon results of 13 week subchronic experiments in rats and mice which showed that doses of 60 mg/kg/day or higher produced damage to renal tubular epithelium in rats. Also, at 50 mg/kg/day and higher, there was a break in linear pharmacokinetics whereby excretion did not remain proportional to intake, suggesting a saturation of renal excretory capacity.

No treatment-related effects on animal survival, clinical observations or gross pathological findings occurred at either the 52 or 104 week sacrifices. However, in high-dose females, there were significant decreases in bodyweight gains at both intervals.

There were compound-related increases in histopathological lesions in 2 tissues. At all dose levels except 1 mg/kg/day, there was an increase in brown tubular cell pigment in the kidneys of both males and females. At levels of 15 mg/kg/day and 45 mg/kg/day in males and 45 mg/kg/day in females there was also an increase in renal pelvic microcalculi and there was a slight increase in renal pelvic transitional cell hyperplasia in the 45 mg/kg/day females. The hyperplasia was considered secondary to the microcalculi.

The nature of the brown pigment was not determined. However, all of these kidney changes occur spontaneously in aging control animals, and it is questionable whether the increases observed represent potential safety concerns at the levels of human exposure.

A summary of all tumor incidences is presented in Appendix 1. The only neoplastic finding of concern was an increase in astrocytomas in the brains of high-dose male rats. There was no increase in treated females. Based upon

the standard 3 coronal brain sections examined. (forebrain, midbrain, and hindbrain) the incidence of astrocytomas in male groups was as follows:

Table 5. Initial astrocytoma incidence in male rats.

Control	1 mg/kg	5 mg/kg	15 mg/kg	45 mg/kg
1/60	0/60	0/60	2/58	4/60

The tumor in a control male had the earliest onset at 21 weeks and apparently was responsible for death of the animal. No gliomas were found in the spinal cords of male rats.

Because of the greater incidence of tumors observed in high-dose animals, the remaining preserved brain tissues from all animals were sectioned, giving a final total of 6-8 brain sections per animal. Two more astrocytomas were found in high-dose male rats in the additional brain tissue. No further tumors were found in other male groups. One additional astrocytoma was found in a 5 mg/kg group female. The final over-all incidence of astrocytomas in males was, as follows:

Table 6. Final astrocytoma incidence in male rats.

Control	1 mg/kg	5 mg/kg	15 mg/kg	45 mg/kg
1/60	0/60	0/60	2/58	6/60

Statistical analysis of adjusted data using a 1-tailed Fisher-Irwin Exact test indicated the incidence of astrocytomas in male rats was increased in the high-dose group ($p=0.05$). Also, a Cochran-Armitage Test indicated a statistically significant positive trend ($p<0.01$; Hazleton, 1986). Based upon these statistical analyses, the contracting laboratory report states "While the characteristics of these tumors did not conform to published characteristics of chemically induced brain tumors, this finding is, nevertheless, suggestive of a possible carcinogenic effect at a dose of 45 mg/kg/day" (Hazleton, 1986).

It is generally accepted that statistical analysis alone should not be the basis for interpreting a biological experiment (Interdisciplinary Panel on Carcinogenicity, 1984; OSTP, 1985). There are many non-quantitative, biological factors to be considered when assessing the evidence for a carcinogenic effect in animals. These include:

1. High variability of spontaneous incidence in control animals of the same strain in concurrent and earlier studies (historical controls).
2. Decreased time-to-tumor development (latency) in treated vs. control animals.
3. A dose-related increase in incidence in more than 1 experimental group or in both sexes.
4. A greater degree of tumor growth (neoplastic progression) in treated vs. control animals.
5. The presence of preneoplastic or toxic lesions in the putative target tissue.
6. The presence of multiple tumors in the putative target tissue.
7. Positive genotoxicity of the test substance.

These factors apply to evaluation of suspected carcinogens at any site, including the central nervous system, as discussed by Koestner (1986) and by Ward and Rice (1982).

The characteristics generally attributed to a brain carcinogen were not present in this experiment. There was no evidence of decreased tumor latency, the increase was limited to high-dose males, no preneoplastic lesions such as gliosis were present in treated animals, all tumors were solitary, and the tumors in treated animals were not larger or more anaplastic than generally seen in control animals. In fact, the largest and most lethal tumor was the one in a control male. There is a final consideration, i.e. most, if not all known brain carcinogens show clear genotoxicity in mutational assays (Federal Register, 1983 and Kleihues *et al.*, 1982), whereas 2,4-D is negative in most such assays.

The absence of preneoplastic and toxic lesions in the brains of the high-dose male rats warrants particular attention. Generally, when a group of inbred test animals is exposed to a carcinogenic level of a chemical throughout the course of an experiment, the different stages of carcinogenicity are repre-

sented in many of the animals. It would be unusual for 6 animals to exhibit fully developed tumors while the remaining 54 fail to develop even the earliest stages of neoplasia or other signs of toxicity in the putative target cells.

All of these considerations do not, however, totally preclude the possibility that 2,4-D may be a weak neurocarcinogen. Although known animal neurocarcinogens are either relatively potent, genotoxic chemicals or oncogenic viruses, the central nervous system could be a target tissue for relatively weak promotional or indirect carcinogenic effects as reported in other tissues. Pathological evidence of neurotoxicity has not been identified; however, clinical signs of neurotoxicity have been reported in animals exposed to high levels of 2,4-D, indicating that the chemical may functionally affect the central nervous system (See section 3). Since little is known of neurocarcinogenic mechanisms, further consideration of this area would be speculative.

The over-all incidence of brain tumors of similar type (gliomas) reported in 2,320 historical control male Fischer 344 rats up to 116 weeks of age in the U.S. National Toxicology Program (NTP) was 0.8% (Solleveld et al., 1984). Among 529 animals allowed to live out their life-span (median age 28 months) the incidence was 2.9%, indicating an increase in incidence with advancing age. If one assumed there was no compound-related effect in the 2,4-D study, the over-all glioma incidence in male rats, combining all groups, was 3.6%. This is considerably higher than the NTP figure for 2 year studies, and slightly higher than the incidence for control rats with a median age of 28 months. It must be pointed out; however, that historical control data is generally based upon 3 brain sections per animal rather than the 6-8 examined in the 2,4-D study. Since most gliomas in rat brains are found only upon microscopic examination, the number of tumors reported is probably a function of the amount of brain tissue examined histologically. Thus, the 4 tumors initially found in 2,4-D treated high-dose male rats may be more appropriate for comparison to historical control rates. This would give an over-all glioma incidence in male rats of 2.8%.

The interpretation of animal tumor data where there is high variability in tumor incidences among different control groups must take into consideration the possibility of statistical false positive findings. Solleveld et al. (1984) report that astrocytomas in Fischer rats are one of the tumor types in the NTP testing program that exhibit statistically significant intergroup variability.

7.2. Industry Task Force 2,4-D Mouse Study

Groups of 60 male and 60 female weanling B6C3F1 mice were treated with 2,4-D (Acid, 97.5% pure) in the diet at levels of 0, 1, 15 or 45 mg/kg body weight/day for 106 weeks (Hazleton, 1987).

No treatment-related effects on animal survival, clinical observations, bodyweights or gross pathological findings occurred. The only histological alteration found to be treatment-related was increased cytoplasmic homogeneity of renal tubular epithelium in male mice receiving 15 mg/kg body weight/day and 45 mg/kg body weight/day. Untreated and low-dose animals had cytoplasmic vacuoles in the epithelium. The significance of this finding is uncertain.

A summary of all tumor incidences in the mouse study is presented in Appendix 2. There were no treatment-related increases at any site.

In summary, there was no clear evidence of toxicity as the result of exposure to 2,4-D under the conditions of the experiment, and no carcinogenicity was evident. One may question whether a maximum tolerated dose was employed in the mouse study. However, the doses administered were 600-fold higher than the maximum reported human exposure (Libich et al., 1981).

7.3. Evaluation of Pathology and Carcinogenicity

Two recent animal experiments with rats and mice conducted for the Industry Task Force on 2,4-D Research Data were adequate to detect potential chronic toxicity or carcinogenicity. The only finding of concern was an increase in the incidence of astrocytomas in the brains of male rats administered 45 mg/kg/day of 2,4-D for 104 weeks. There is insufficient evidence to conclude that these tumors were related to 2,4-D exposure. Although there was a statistically significant increase in treated animals, an assessment of biological factors suggests the tumors were spontaneous rather than compound-related.

8. EPIDEMIOLOGY

The available epidemiological studies of persons potentially exposed to 2,4-D include cohort and case control studies. In cohort studies, the experience of exposed individuals is followed in comparison with an unexposed group often drawn from the general population. If the exposure is well characterized the incidence of a number of different diseases in relation to exposure can be assessed. Often mortality may be used as a substitute for incidence. In case control studies, individuals with specific diseases are iden-

tified and comparable controls and their past exposures ascertained, usually by means of interviews. As these two different classes of studies have different methodologic aspects and potential concern for biases, they will be considered separately. It should also be pointed out that the classification of soft-tissue sarcomas is controversial. As it was not possible to review the data upon which tumors in the epidemiological studies were classified, the reports of the authors have been taken as published. The findings of these studies are summarized in Table 7 and their salient points discussed in the following section.

Table 7. Summary of epidemiology studies.

<u>GROUP</u>	<u>FINDINGS</u>	<u>REFERENCE</u>
COHORT STUDIES		
Employees manufacturing 2,4-D and 2,4,5-T in Denmark. Two groups sized 3,844 and 615 exposed from 1947/1951 to 1982	Positive: Four cases of soft tissue sarcoma (STS) with only 1.09 expected. Negative for malignant lymphomas.	Lynge, 1985.
A group of 348 Swedish railway workers who used several herbicides for at least 40 days/year.	No indication of STS or non-Hodgkins lymphoma (NHL). Lacked power to indicate or exonerate 2,4-D.	Axelsson and Sundell, 1974 and Axelsson <i>et al.</i> , 1980.
A group of 1,971 male herbicide applicators from Finland exposed to herbicides for >2 weeks/year from 1955 to 1971.	No excess of cancer, no cases of NHL or STS.	Riihimäki <i>et al.</i> , 1982.
A group of 1,222 Ontario Hydro sprayers in which exposures to phenoxy herbicides were high, had worked in the forestry trade for >6 months between the years 1950-1982.	No cases of NHL or STS have yet been identified but latent period short and group size was small.	Green, 1986.
A group of 354,620 Swedish agricultural or male forestry workers born between 1891 and 1940 compared to a reference cohort of ca. 2,000,000 men with other occupations.	In men working in land and/or animal husbandry, 253 cases of STS were observed with a relative risk of 0.9. In timber cutting, 49 cases observed with a relative risk of 1.0. A large study with sufficient power but with poor identification of exposure.	Wiklund and Holm, 1986.
CASE CONTROL STUDIES		
Group exposed to phenoxy herbicides in agriculture and forestry from 1950 to the mid 1970s in Sweden	Relative risk for STS of 5.3.	Hardell, 1981; Hardell and Sandstrom, 1979.
South Swedish group of workers exposed to phenoxy herbicides.	Relative risk for exposure to all phenoxy herbicides of 6.8 for STS. Relative risk for 2,4-D was 4.2.	Eriksson <i>et al.</i> , 1981.

Swedish workers exposed to herbicides and chlorophenols.	Relative risk of malignant lymphoma with exposure to phenoxy herbicides or chlorophenols was 6 and for exposure to phenoxy herbicides alone, 4.8. No dose response relationship for phenoxy herbicide exposure.	Hardell <u>et al.</u> , 1981.
New Zealand study on workers exposed phenoxy herbicides > 1 d/year.	Non significant risk for STS of 1.3 and 1.5 for NHL but information on exposure may not be accurate.	Smith <u>et al.</u> , 1984.
A group of Vietnam veterans who were possibly exposed to various phenoxy herbicides in between 1962 and 1971.	Odds ratio (OR) of 0.53 for Vietnam service, OR of 0.7 for exposure to Agent Orange. Short observation period may limit power.	Greenwald <u>et al.</u> , 1984.
STS in men and women in an area in Northern Italy.	Exposure included 2,4-D, MCPA and 2,4,5-T. No excess risk of cancer associated with phenoxy herbicide exposure in men. Among living women relative risk was 2.7 (90% CI 0.59-12.37). In women alive at time of interview, <75 years old, exposed in 1950-55 period, age adjusted odds ratio was 15.5 (90% CI 1.3-180.3). The study was of low power.	Vineis, <u>et al.</u> , 1987
60 cases of primary mesothelial ovarian tumors and 127 living controls with non-ovarian malignancies.	Cases and controls from same hospital based cancer file. Herbicide exposure determined as definite if the subject or next of kin described personal use of herbicides, probable exposure if a farmer resided in areas with known herbicide usage. Eight cases gave a definite history, no controls, combining definite and probable, relative risk for herbicide exposure was 4.38 (1.90-16.07). Series relatively small and recall bias cannot be excluded. Study not informative in terms of 2,4-D exposure.	Donna <u>et al.</u> , 1984

Conducted on male cases with STS, Hodgkins Disease (HD) and NHL from Kansas (139 STS, 132 HD, 172 NHL).

No significant association between farming and/or herbicide use and STS or HD. For NHL and farming the OR was 1.4. Farm herbicide use on any of wheat, corn, sorghum, or pasture gave an OR of 1.6 for NHL. Increasing risk of NHL with increasing duration of herbicide use and/or increasing time since first exposure. Higher risk also noted with greater herbicide use without protective equipment. Use of 2,4-D was not determined directly and exposure to other chemicals may confound analysis.

Hoar et al.,
1986a and
1986b

Case control study in Western Washington State including 128 cases of STS, 575 of NHL and 694 population controls.

All data obtained by personal interview. No excess risk for past occupational exposure to phenoxy herbicides for either STS or NHL. Elevated risk of NHL among men who had been farmers (RR of 1.33: 95% CI 1.03-1.7), forestry herbicide applicators, 4.8 (95% CI; 1.2-19.4) and those potentially exposed to phenoxy herbicides for 15 years or more during the period prior to 15 years before cancer diagnosis 1.71 (95% CI; 1.04-2.8). Also increased risk of both STS and NHL in individuals reporting prior occurrence of chloracne.

Woods, et
al., 1987

8.1. Cohort Studies

In spite of the apparent superiority of the cohort design in many aspects, cohort studies have many deficiencies which makes them less informative than case control studies. One of the important reasons for this is that cancer such as STS and NHL are relatively rare compared to other cancers and also probably occur only after a long latent interval. A cohort study therefore has to be very large and the observation period very prolonged for a negative finding, a feature of the majority of them, to be of much assurance. In addition, there are potential difficulties with regard to exposure, though these are shared with case control studies.

One class of cohort study which has been used in the interim evaluation of the potential for phenoxy herbicides to increase cancer risk is identification of individuals in occupations with potential exposure to these substances such as agricultural and forestry workers. Because exposure is not individually identified in such studies, substantial obscuring (dilution) of potential effects

may occur. The value of these studies is very small and, with one exception, studies using occupation to indicate exposure have been ignored. However, many of the other cohort studies suffered from a similar deficiency, in that, even though based on specific groups of workers, details of individual exposure were unknown. An example of this is the recent Ontario Hydro Study (Green, 1986).

The other major difficulty is that nearly all cohort studies, at least in part, depended on review of historically available records with follow-up of identified individuals to the present day. Such historical cohort studies present major difficulties in the identification of individual exposure, particularly in terms of intensity. Thus it is very difficult to evaluate a dose response relationship, a critical consideration in human carcinogenesis. Although some of the cohort studies have incorporated a protocol for follow-up, in nearly every instance this has been of short duration and has not helped to achieve much resolution of the exposure issue.

In common with the case control studies, there are few groups who are known to have been exclusively exposed to 2,4-D. In the majority of instances, exposure has been to 2,4,5-T as well, often in excess of 2,4-D and, even in a positive study, it may not be clear which agent was potentially responsible.

Potentially the most informative of cohort studies are those on workers involved in the manufacture of specific chemicals in specific plants. The available studies of this type in the United States have only reported on workers involved in the manufacture of 2,4,5-T and are therefore not considered further. There has; however, been one study of this type from Denmark (Lyngé, 1985) in which 3,844 workers in one plant and 615 in another, employed from 1947 and 1951 respectively were followed to December 31, 1982, by linkage with the Central Population Register for Death and the Danish Cancer Register. Exposure appeared to be largely to 2,4-D or other chemicals of a non-phenoxy type, although a small amount of 2,4,5-T was made between 1951 and 1959 in the larger plant. In this study an excess of soft tissue sarcoma was found. Restricting attention to those where the latent period exceeded 10 years, there were 4 observed cases with 1.09 expected (95% confidence intervals [CI] of 1.00-9.39). A corresponding analysis for malignant lymphomas indicated 4 observed, compared to 3.04 expected, a non-significant difference. This is the only cohort study which has shown an excess of soft tissue sarcomas, but it is also one of the largest which had relevant exposures.

Part of the difficulty in interpretation is the exposure to 2,4,5-T and, for this reason, it cannot be concluded that the excess was due to 2,4-D.

All the other cohort studies so far reported were negative. One of the earliest was the study of Axelson (Axelson and Sundell, 1974; Axelson, et al., 1980). This was a small cohort study of Swedish railroad workers, who were selected for having been exposed on at least forty days (per year) to herbicides. Although an initial apparent excess of tumors in those exposed to another unrelated herbicide (amitrole) was reported, this finding was reversed in the second report. However, a non-specific excess of tumors in those exposed to phenoxy herbicides or phenoxy herbicides and amitrole was noted (two stomach cancers, vs. 0.33 expected in the group with a latency of 10 or more years). There were no soft tissue sarcomas (STS) or non-Hodgkins lymphoma (NHL) in this group. This study only involved 348 persons, and lacked the power to clearly indicate the role of herbicides in the causation of stomach cancer as these could have arisen by chance.

Riihimaki et al., 1982, reported on 1,971 males who were exposed to 2,4-D and 2,4,5-T as herbicide applicators in Finland for at least two weeks per year from 1955 to 1971. They were only followed for nine years through to 1980 and no excess of cancer with no cases of STS or NHL were noted.

In the Ontario Hydro Study (Green, 1986), the numbers of exposed workers was also small. However, exposures to phenoxy herbicides were known to have been high. This study is potentially of importance, not so much in the fact that it has been negative. (no cases of NHL or STS have yet been identified) but because it has the potential for further prolonged follow-up. However, in common with other cohort studies, exposures were to other herbicides in addition to 2,4-D and it would be unable to specifically identify a risk related to 2,4-D.

The work of Wiklund and Holm (1986) is of note because it relates to a very large cohort of 354,620 men born between 1891 and 1940 and described as agricultural or forestry workers in the Swedish population and housing census of 1960. These men, together with a reference cohort of nearly two million men with other occupations, have been followed by linking to the Swedish Cancer Environment Registry for 1961 through 1979. The cohort was sub-categorized according to presumed exposure to phenoxy herbicides by the named occupations. Large numbers of STSs occurred but, in the final analysis, there was no significant excess in any of the sub-groups. For example, in those working in land and/or animal husbandry, 253 cases were observed with

a risk of 0.9 relative to the control population (95% CI: 0.8-1.1). Similarly, for timber cutting, 49 cases were observed with a relative risk of 1.0 (95% CI: 0.7-1.3). Both these groups had presumed exposure to 2,4,5-T and 2,4-D. Although this was a large study with sufficient power to identify the increased incidence of STS (excesses) noted in the case control studies conducted in the same country, there was undoubtedly some dilution effect because of the characterization of workers by job titles. There was also a potential shortage of person years of risk for periods where an excess might be expected to occur. Nevertheless, the authors have calculated that, even with dilution, their studies should have been capable of detecting a relative risk of 1.5 which they believe would have been compatible with the relative risks in excess of five noted in the Swedish case control studies (considered below). In many respects this report can be regarded as a reasonably powerful negative study. The study has the potential to further resolve the issue, particularly if further details of exposure are obtained (possibly through a case control approach which is reported to be in the planning stage).

In summary therefore, the majority of cohort studies are of limited usefulness in terms of their size and therefore relative lack of power as well as their difficulties in characterizing exposure. The one positive cohort study, that of Lynge (1985) has to be given substantial weight, even though the only excess noted was of soft tissue sarcoma. However, it cannot be used to indicate that the excess was due to 2,4-D alone.

8.2. Case Control Studies

The case control studies have been largely responsible for identifying potential risks of cancer following phenoxy herbicide exposure in man and have so far been reported from Sweden, New Zealand, Italy and the United States. Swedish studies pointed to potentially important relative risks in (>5) for STS and NHL. The studies in New Zealand have been negative or shown small and nonsignificant excess of these two tumors. Of the studies conducted and so far reported in the United States, one was negative but two were positive in relation to NHL.

Before proceeding to a detailed review of each study, a comment on issues of methodology is in order. Substantial criticism has been levelled against many of the case control studies, particularly those from Sweden, because of the possibility for bias in the recall of information. It is accepted that recall bias is possible in any case control study (a bias in which cases are

more likely to recall relevant exposures than controls because they have the disease and the incentive to try and find a reason for developing it). Because of the potential for recall bias in any case control study, considerable attention is paid to specific points of detail in order to ensure that it is minimized. Although preferable, this is not always achievable. Interviewers who collect the information should be kept unaware (blinded) as to case and control status, questions should be asked in a pre-assigned format and those relating to specific exposure be only part of a series of questions relating to exposure to a number of different substances. The reports of the Swedish case control studies suggest that sufficient care may not have been taken on these points. Hardell (1981) addressed this issue specifically and, in addition, discussed a suggested bias apparently first proposed by Dr. Philip Cole in testimony in 1980 to the Environmental Protection Agency of the United States. His suggestion was that cases might not always directly record employment in agriculture or forestry, but first remember exposure to phenoxy herbicides and then, conditionally on that recall, also remember earlier jobs. Such a bias might invalidate some of the approaches taken by Hardell and his colleagues to obtain detailed occupational exposures. Hardell (1981) claimed that this putative bias was largely discounted by an investigation of colon cancer conducted using the identical approach as in his other studies. In this study a risk related exposure to phenoxy herbicides was not identified; however, this may not discount the possibility of a bias as discussed further below.

In any observational epidemiologic inquiry a systematic bias may occur. There may be a systematic bias in the Swedish case control studies which persists throughout all the studies as they were essentially performed using the same technique. However, there may also be a systematic bias in the New Zealand case control studies which has led to those studies tending to be negative. In both sets of studies it was impossible to be certain of the nature of the exposure the cases and controls encountered. However, in view of the high relative risks observed in the Swedish studies, they have to be considered seriously in the present context.

Hardell and Sandstrom (1979) reported a case control study of STS which arose from the observation by Hardell in a series of cases under his care that exposure to phenoxy herbicides appeared to be reported more frequently than expected. The case control study largely confirmed this observation with a relative risk of 5.3 in those exposed to phenoxy herbicides in agriculture and

forestry from 1950 to the mid 1970s. A further analysis conducted by Hardell (1981) confirmed this finding.

In a second study in South Sweden using a completely different case series, Eriksson et al (1981) found a relative risk of STS for exposure to all phenoxy herbicides of 6.8 (95% CI: 2.6.-17.3). It was possible to characterize exposure to non-2,4,5-T phenoxy herbicides including 2,4-D where the relative risk was 4.2. In these studies, as in the other Swedish case control studies, cases were identified from the Tumour Registry and were pathologically confirmed. For live cases, live controls were identified from the Population Registry and for dead cases, dead controls from the Death Registry. Each case was matched with 2 controls. Exposure data were obtained from a mailed questionnaire with questions relating to phenoxy herbicides as part of a series of questions relating to exposure to specific chemical substances. Where there was doubt over the answers received the answers were supplemented by telephone interviews conducted blind according to case or control status. With this design, the extent to which recall bias will occur, is dependent on the extent to which telephone interviews were indeed used when answers were uninterpretable. If there was a tendency to use more telephone interviews for cases than controls and this occurred in all studies then a systematic bias could in fact have occurred, leading to elevated relative risks. The negative finding in the case control study of colon cancer (Hardell, 1981) is to a certain extent reassuring, providing the use of telephone confirmation was not less for the colon cancer cases than for the STS or NHL cases. Also reassuring is the fact that when analyses were restricted to the data obtained from the mailed questionnaire, similar relative risks were obtained. Hardell et al. (1981) used the same method to evaluate malignant lymphoma. Once again, the study arose from the observation of a case series where exposure to phenoxy herbicides appeared to be greater than expected. The cases in this study possibly included the case series which prompted the hypothesis and included 60 cases of Hodgkins Disease and 105 of NHL, together with four unclassifiable lymphomas for a total of 169 cases with two controls, each derived in the same method as for the earlier studies. Relative risk for exposure to phenoxy herbicides or chlorophenols was 6 (95% CI: 3.7-9.7); for exposure to phenoxy herbicides alone, 4.8 (95% CI: 2.9-8.1). There was no dose response relationship for phenoxy herbicide exposure.

The studies in New Zealand were conducted because of the Swedish observations and because phenoxy herbicides are extensively used in that

country. In each instance, the cases and controls were drawn from the National Cancer Registry. Both cases and controls were restricted to those from public hospitals as there appeared to be some difficulty in contacting and interviewing the relatively small numbers of cases in private hospitals. Data was derived from telephone interviews of cases and controls. The interview schedule, though not given in any of the papers, appeared to be less extensive and potentially less specific in terms of phenoxy herbicide exposure than the schedule used in Sweden. This might contribute to a negative finding though the authors claim that their use of cancer controls tends to overcome the potential problem of recall bias in Swedish studies.

The first study related to STS (Smith et al., 1984) in which there were 82 cases and 92 controls. The relative risk for those potentially exposed to phenoxy herbicides of one day (per year, but not in the five years before cancer registration) was 1.3 (90% CI: 0.6-2.5). In the second study (Pearce et al., 1986), NHL was evaluated by a similar design. There were 86 cases and 228 population controls. A relative risk of contracting NHL of 1.5 (95% CI: 0.8-2.7) was observed for those using any agricultural spray. The relative risk was 1.3 (95% CI: 0.8-2.1) for cancer controls and 1.6 (95% CI: 0.3-3.1) for population controls for those who ever sprayed an agricultural chemical involving exposure to phenoxy herbicides, including both 2,4,5-T and 2,4-D.

These studies clearly do not demonstrate the absence of risk. There is a small, though not significant, elevation of risk for both diseases and the confidence intervals would not exclude a relative risk of two or more, depending on the case series. If an effect were really present, it is likely that the risk may have been underestimated because of the difficulty of obtaining exposure information.

In the United States, Greenwald et al. (1984) attempted to evaluate whether Vietnam service had increased the risk of STS by identifying cases from the New York Tumour Registry. Population controls for living cases were obtained from driver licence files and controls for diseased cases from the Death Certificate Register for men with potential Vietnam service i.e. those aged eighteen to twenty-nine, between 1962 and 1971. The cases had to have been diagnosed between 1962 and 1980. Data were obtained from personal interviews of cases or next of kin. The odds ratio for Vietnam service was only 0.53 and, for reported exposure to Agent Orange, 0.70 (95% CI: 0.17-2.92). Agent Orange is a 1:1 mixture of 2,4,5-T and 2,4-D. This study therefore showed no association though the width of the confidence intervals indicates

that it was not powerful enough to exclude an elevation of risk approximating to three. Further, the analysis appears to have ignored the issue of latency and may not be an appropriate test of the hypothesis.

Reports of two case control studies have appeared from Italy. Both relate to potential exposure to phenoxy herbicides in women, in the one case to STS and, in the other, to ovarian tumors.

Vineis, *et al.* (1987) studied soft tissue sarcomas diagnosed in men and women in an area in Northern Italy where rice is grown. Women are traditionally employed as rice weeders and during a period commencing in 1950 they were exposed to increasing amounts of phenoxy herbicides that were being used initially to experimentally control weeds. This exposure included 2,4-D, MCPA and 2,4,5-T; however, use of the latter was banned in 1970.

The case series was identified in 1981-1983 and a random sample of controls was drawn from the population. In addition, diseased subjects were chosen as controls for the 37 of the 135 cases who had died at the time of interview. No excess risk of cancer associated with phenoxy herbicide exposure was found for men. Among living women the relative risk was 2.7 (90% CI 0.59-12.37) but when attention was restricted to women alive at the time of interview, less than 75 years of age, exposed in 1950-55 period an age adjusted odds ratio of 15.5 (based on only 15 cases with a 90% CI of 1.3-180.3) was determined. Similarly when exposure to phenoxy herbicides among living females who had regular jobs in agriculture was considered the age adjusted odds ratio for women known to be exposed was 3.

Although this study is of low power it suggests an excess risk of STS in women known to have been occupationally exposed to phenoxy herbicides.

Donna, *et al.* (1984) studied a series of 60 cases of primary mesothelial ovarian tumors and 127 living controls with non-ovarian malignancies, drawn from the same hospital based cancer file. Ten of the cases of ovarian tumors were dead and next of kin were interviewed. These cases were studied because of preliminary data suggesting that some of the herbicides widely used in the rural areas from which the cases were drawn were carcinogenic in animals. Herbicide exposure was determined as definite if the subject or next of kin described personal use of herbicides and was familiar with the various commercial brand names, probable exposure if a farmer resided in areas where there is known herbicide usage. Eight cases and no controls gave a definite history of exposure combining definite and probable, the relative risk for herbicide exposure was 4.38 (1.90-16.07).

This was not a population based study. The case series was relatively small, controls were drawn from a cancer file, little detail is given. It is possible that there was an undue emphasis on herbicide exposure so recall bias cannot be excluded, even though the authors claim that it is unlikely with a cancer control series.

However, there was no attempt to characterize herbicide exposure according to nature of agent and thus this study is relatively noninformative in terms of 2,4-D exposure.

A recent case control study (Hoar et al., 1986a and b) conducted by a group from the environmental epidemiology branch from the U.S. National Cancer Institute, was designed to avoid many of the potential methodologic problems of other case control studies. The study was conducted in Kansas, known to be a wheat producing area and where herbicides were used more frequently than insecticides. Of the herbicides, 2,4-D was more commonly used; 2,4,5-T was also used "along with myriad other chemicals".

It was decided to study male cases with STS, Hodgkins Disease (HD) and (NHL). Cases were identified through the University of Kansas Cancer Data Service which is a population based registry covering the state of Kansas for the years 1976 to 1982. There were 200 males with STS; 173 with HD; 297 with NHL of which 200 were selected at random for study. A pathology review was conducted with confirmation of the diagnosis in 81%, 85% and 90% of the cases respectively (139 STS, 132 HD, 172 NHL). These appear to have been regarded as the eligible cases for the study. Three controls per case were selected by random digit dialing for those less than 65 years of age and from medicare files for those more than 64 if the corresponding case was alive. If the case was dead, controls were selected from Kansas State mortality files. Controls were matched to the case by age (plus or minus two years) and vital status and, for the deceased controls, by year of death of the case. A deceased individual was not eligible to be a control if they died of a STS, HD, NHL or a malignancy of an ill-defined site. Deaths resulting from homicide and suicide were also excluded.

Half of the cases of STS and NHL and one third of the cases with HD had died before the study commenced. Their next of kin were interviewed, as were the corresponding deceased controls. All interviews were conducted by telephone and were completed in 96% of the eligible STS cases, 92% of the eligible HD cases, 99% of the eligible NHL cases and 94% of the controls.

In the analysis, individuals were considered as "farmers", if they had reported as "having worked or lived on farm-land". An unmatched analysis was used as a "matched analysis yielded results similar to those provided by the unmatched approach". The same set of controls were used for analysis of all sites although, as the major findings were restricted to Non-Hodgkins Lymphoma, the detailed analyses were conducted by comparing that set of cases with all controls. No significant association was found between farming and/or herbicide use and STS or HD. Significant associations were found with NHL and further discussion will be restricted to these.

For NHL there was an association of borderline significance for farming (odds ratio (OR) of 1.4, 95% CI: 0.9-2.1). Farm herbicide use on any of the four specific crops (wheat, corn, sorghum, or pasture) was reported by 40 cases with NHL compared to 192 controls, for an OR of 1.6 (95% CI: 0.9-2.6). There was a significant trend in risk of NHL with increasing duration of herbicide use to an OR of 2.0 for sixteen or more years use, for frequency of herbicide use (with an OR of 6.0 for twenty-one or more days use per year). Risk of NHL also increased with time since first exposure, the greatest risk being found for farmers who started using herbicides before 1946 (OR of 3.3). This trend was diminished by controlling for frequency of herbicide use but, after this control, farmers who began use before 1946 still had an excess risk (OR of 2.2). This latter trend is reported only in the text, numbers are not provided and it is not indicated whether the elevated risk for use before 1946 is significantly different from the risks for use between 1946 and 1965 which was 1.7.

Subjects who usually mixed or applied herbicides themselves had increase risk of NHL with an OR of 1.9 (95% CI: 1.1-3.3). Among those who did not mix herbicides, the OR was 1.1 i.e. not elevated. Higher risk was also noted with greater herbicide use without protective equipment.

This appeared to be a well conducted study although, as for any study, some questions can be raised. Phenoxy herbicide use was said to be synonymous with 2,4-D use. However, the questionnaire did not address dates and frequency of use of each specific herbicide though, given the nature of the data collection including information obtained from proxies of both cases and controls (by design), it seems unlikely that detail of this type could have been anticipated. There has to be concern with the possibility of recall bias, associated with knowledge of the disease in cases. Indeed it is possible that, at the time the study was conducted there already was some information available

to farmers and next of kin of the possible association of herbicide use with cancer from the reports in Sweden. With the use of proxy cases and controls, the opportunity for misclassification of exposure was substantial although, in general, this would normally be expected to reduce risks towards zero and not produce spurious increases in risk.

Although the authors clearly indicate that the study is supportive of the possibility that 2,4-D was the responsible agent for the NHL excess, as pointed out by MacMahon (1986, in a review conducted for the U.S. EPA) previous studies had suggested that all three tumors were likely to show increased risk and this study only shows increased risk for one of them. However it is difficult to perceive of recall bias preferentially applying only to NHL. Also, the authors did not analyze data on individuals exposed only to 2,4-D and not other chemicals.

Nevertheless, it is questionable as to whether 2,4-D could be regarded as the responsible agent. Apart from the fact that the estimates of phenoxy herbicide exposure may not reflect use of 2,4-D alone, it is possible that exposure in the early part of the historical period covered by this study was to substances such as 2,4,5-T, likely to be contaminated with more toxic dioxins. That contaminated herbicides may be responsible is suggested by the highest risk for those exposed prior to 1946. However, it is not clear yet whether this risk is in fact significantly different from the risk in the succeeding twenty year period. The risk from recent exposure would be expected to be lower than for early exposure, possibly because of greater care in use as well as lack of expiration of the relevant latent period.

Woods, et al. (1987) conducted a population based case control study in Western Washington State that included 128 cases of STS, 575 of NHL and 694 population controls. All data were obtained by personal interview. There was no excess risk for past occupational exposure to phenoxy herbicides for either STS or NHL. However, there was an elevated risk of NHL among the following groups: Men who had been farmers with a relative risk of 1.33 (95% CI 1.03-1.7), forestry herbicide applicators, 4.8 (95% CI; 1.2-19.4) and those potentially exposed to phenoxy herbicides in any occupation for 15 years or more during the period prior to 15 years before cancer diagnosis 1.71 (95% CI; 1.04-2.8). Although these risks came from sub-group analyses, the sub-groups were evaluated because of positive findings in other studies and because of knowledge on latent period effects in relation to carcinogenicity. The significant excesses are compatible with the expectations from other studies are

therefore important. An additional confirmation was that increased risk of both STS and NHL was observed among those individuals reporting prior occurrence of chloracne. This presumably indicates either those who had severe exposure or might have been unduly susceptible to the toxic effects of phenoxy herbicides.

The authors also discussed why lower risks might be found in studies in the United States compared to Sweden. They point out that exposures in Sweden tends to be concentrated over a much shorter time period than for herbicide exposure in the United States. They have calculated a mean maximum daily dose of 45 ug/kg for American workers exposed to 2,4,5-T compared to mean of 90 ug/kg for Swedish workers. They also considered the possibility that Scandinavians might have undue susceptibility to the effect of phenoxy herbicides. They found that when their analysis was restricted to persons from Scandinavia only, the risk estimates for STS in relation to past occupational chemical exposures were substantially greater than those observed among the study population as a whole. This was true both for high level phenoxy herbicide exposure (relative risk of 2.8, CI; 0.5-15.6) and for high level chlorophenol exposure. This analysis was; however, based only on 15 STS cases.

This seems to have been a carefully conducted study and, although overall no increased risk was found for phenoxy herbicide exposure and STS or NHL, the sub-groups where increased risk was demonstrated are compatible with a true biological effect. It is important to recognize that when case control studies are performed in a general population group, it is likely that excesses will only be found among sub-groups who have had the opportunity for relevant exposures at an appropriate time period. This applies even if the area for study has been selected as one where it is known that herbicide exposure occurs.

Another case control study is being conducted by the Environmental Epidemiology Branch of the National Cancer Institute in Iowa and Minnesota involving 594 Leukemias, 690 cases of NHL and 1,245 controls (Cantor and Blair, 1986). Preliminary results indicate no overall increased risk for NHL associated with living or working on farming. However, persons reporting the use of 2,4,5,-T had a two-fold risk of NHL (OR = 2: 95% CI; 0.7, 5.2) while those using 2,4-D had only a slightly elevated risk (OR = 1.2: CI; 0.9, 1.8). Unfortunately, information was not gathered on the number of days per year of pesticide use. Since this was the variable that showed the strongest as-

sociation risk of NHL in the study of Hoar, et al. (1986), the investigators have decided to recontact subjects to try and obtain this information.

8.3. Evaluation of Epidemiology Studies

An important feature of studies of potential carcinogenicity is consistency. The difference between the results in New Zealand and Sweden indicates lack of consistency; however consistency has been observed in the finding of an excess of STS in the studies in Sweden and Italy using a case control approach and in Denmark in a cohort approach. Further consistency has been observed in finding an excess of NHL in Sweden and in the United States in case control approaches. Although it is clear that 2,4-D cannot be exonerated as a reason for the excess, especially from the results of the Danish cohort study and the U.S. case control study, neither can these single studies, of themselves, be used to classify the risk as being confirmed.

Using IARC terminology, it may be concluded that there is limited evidence of carcinogenicity in man from exposure to phenoxy herbicides. In terms of exposure to 2,4-D specifically, the evidence must still be regarded as inadequate to classify it as a carcinogen.

9. RISK ASSESSMENT OF 2,4-D

The panel was of the view that it would be useful to give some indication of the theoretical risk to humans from exposure to 2,4-D if it is assumed that 2,4-D is carcinogenic. The human data are not adequate to support such a calculation because, amongst other limitations, the lack of information of specific human exposures to 2,4-D in epidemiological studies. Accordingly, cancer risk was assessed under the theoretical assumption that the increased incidence of astrocytomas observed in male rats in the Industry Task Force 2,4-D study was caused by exposure to 2,4-D. These estimates are not intended necessarily to be accurate estimates of risk nor should the fact that these calculations were made be interpreted as implying that the panel believes that 2,4-D is a carcinogen.

The theoretical risk of cancer at doses experienced in humans was were obtained by fitting the multistage mathematical dose response model (Crump, 1984) to the data on astrocytomas in male rats. The multistage dose response model (Crump et al., 1977; Crump, 1984) has been widely used by the U.S. Environmental Protection Agency (EPA, 1983) and the U.S. Occupational Safety and Health Administration, (OSHA, 1983a). The multistage model is used to

calculate statistical 95% upper confidence limits based upon an assumed linear relation between 2,4-D and cancer risk (i.e. that additional cancer risk is proportional to dose). Risk estimates to humans were made assuming that a given dose rate expressed in mg/kg/day gives the same risk in animals and humans. This assumption is supported by a recently completed study that compares animals and human data for 23 carcinogens (Allen *et al.*, 1987). Table 8 contains the resulting estimates of risk based upon the estimated human exposures from Table 2.

To help place the theoretical risks estimated for 2,4-D into perspective, cancer risks estimated for exposures to known carcinogens are also tabulated in Table 8. Risks from these carcinogens were computed using methodologies similar to that used for 2,4-D (e.g., a linear relations between exposure and risk was assumed in all cases and risk estimated from animal data were made to apply to humans).

Risks from occupational exposure to inorganic arsenic, ethylene oxide and benzene assume exposures at recently promulgated or proposed OSHA standards (OSHA, 1983b). Risk estimates are provided for an exposure duration commensurate with that estimated for occupational exposure to 2,4-D. The effect of intermittent exposure was accounted for in the same way in estimates for both 2,4-D and non-2,4-D exposures. Risk estimates are also presented for conditions which may not generally be regarded as "risky" by the general public: eating peanut products, having a chest X-ray, spending a week in the Rocky Mountains and smoking a single cigarette.

The methods used to estimate the theoretical risks contained in Table 8 involve many uncertainties and should be viewed only as crude indicators of risk based upon the stated assumptions. Nevertheless, theoretical risks for 2,4-D and from other sources were obtained using similar assumptions and the panel believes that these estimates may provide useful comparisons. These comparisons suggest that, even if 2,4-D were a carcinogen, the risk to persons exposed occupationally would be:

- 1) Considerably less than those for workers exposed to carcinogens at levels recently set by OSHA.
- 2) Less than those from some activities that the general public may regard as safe.

Table 8. Estimated risks per million persons under various exposure conditions.

PART I. Hypothetical risk from exposure to 2,4-D based on the theoretical assumption of rat brain carcinogenicity.

<u>Application</u>	<u>Occupation</u>	<u>Days/year Exposed</u>	<u>Years Exposed</u>	<u>Risks per million</u>
Helicopter	mixer-loader	12	20	0.6
	mixer-flagger	12	20	0.3
	flagger	12	20	0.2
Airplane	mixer-loader	12	20	0.09
	supervisor	12	20	0.003
Packsprayers	handheld right-of-way	60	10	5
	gun sprayer	60	10	5
	roadside sprayer	60	10	8
	mist right-of-way	60	10	8
blower	sprayer			
Hand and tank	commercial sprayer	66	13	0.6
Tractor	farmer	14	25	0.4

PART II. Occupational cancer risks at recently established or proposed OSHA levels.

Inorganic arsenic ^a	240	45	8000
(10 ug/m ³)	60	10	400
Ethylene oxide ^b	240	45	3000
(1 ppm proposed)	60	10	180
Benzene ^c	240	40	3000
(1 ppm proposed)	60	10	190

PART III. Other risks

Eating peanut products ^d (aflatoxin, U.S. average)	11
Having a chest X-ray ^e (Lung cancer)	1.5
Spending a week at 3050 m in the Rocky Mountains ^e (Cancer from terrestrial and cosmic radiation)	0.9
Smoking one cigarette (Lung cancer only) ^f	0.6

^a Risks reported in OSHA (1983b)

^b Risks reported in OSHA (1983a)

^c Risks reported in Crump and Allen (1984)

^d Average exposure to aflatoxin in peanut products obtained from FDA (1979). Risk estimates based on animal data in Wogan (1973), Wogan *et al.* (1974), Nixon *et al.* (1974) and Alfin-Slater *et al.* (1969).

^e Based on linear-quadratic model and exposure estimates contained in the National Academy of Sciences BEIR report (NAS, 1980).

^f Estimated from Doll and Peto (1978).

10. GLOSSARY OF TERMS

2,4-D	2,4-dichlorophenoxyacetic acid.
2,4-DP	2,4-dichlorophenoxypropanoic acid.
2,4,5-T	2,4,5-trichlorophenoxyacetic acid.
Adducts	Chemical compounds, usually between a small highly reactive molecule and a large molecule such as a protein or nucleic acid.
Ames	Usually referring to a test for genotoxicity.
Anaplastic	A process involving a loss of differentiation of cells usually seen in certain types of tumor.
Arochlor ^R	A commercial brand of polychlorinated biphenyl.
Astrocyte	A type of cell found in the central nervous system.
Astrocytoma	A tumor composed of astrocytes, usually in the brain.
BEIR	Biological Effects of Ionizing Radiation.
Biphasic	In two parts or two mechanisms.
Chlorophenol	A phenol in which one or more of the hydrogen atoms are replaced by chlorine.
CHO	Chinese Hamster Ovary, usually referring to cells.
Chromatid	A portion of the chromosome consisting of a strand of DNA attached to the centromere.
CI	Confidence Interval
Ci	Curie, a unit of radioactivity.
CNS	Central Nervous System.
Dermal	Referring to the skin such as in skin toxicity.
Embryotoxic	Toxic to the embryo.
EPA	Environmental Protection Agency of the U.S.
F	Female.
Fetotoxic	Toxic to the fetus.
Fibroblast	A connective tissue cell.
Genotoxic	Expression of toxicity which results in changes in the genetic material.
Glioma	A tumor of the glial cells of the nervous system, as well as any tumor of the brain or spinal cord.
Gliosis	An excess of astrocytes in a damaged part of the central nervous system.
GLP	Good Laboratory Practice, usually referring to a set of guidelines which must be followed in conducting experiments.
Hepatocyte	Liver cell.

Histopathological	Pathology at a microscopic or cellular level.
HD	Hodgkins Disease.
Hodgkins Disease	A malignant condition of the lymph nodes, spleen and lymphatic system.
Hyperplasia	An abnormal increase in the number of normal cells in a tissue.
IARC	International Agency for Research on Cancer.
ICR	Referring to a specific strain of laboratory rats or mice.
IP	Intraperitoneal, into the body cavity.
LD ₅₀	The dose, per animal required to kill 50% of the animals so treated.
LV	Low Volume, usually referring to pesticide sprays.
M	Male.
Macromolecules	Very large molecules, usually of biological origin such as proteins or nucleic acids.
MCPA	2-chloro-2-methylphenoxyacetic acid.
MCPP	2-(2-methyl-4-chlorophenoxy)propanoic acid.
Mesothelial	Pertaining to cells of mesodermal origins.
MF	Male and Female.
Microcalculi	Small abnormal concretions, usually of mineral origins.
Micronucleus	A subcellular organelle in the nucleus which is the site of synthesis of ribosomal RNA.
Microsome	A subcellular organelle usually associated with metabolism.
Myotoxic	Toxic to muscles.
NDMA	<u>N</u> -nitrosodimethylamine.
Neoplasm	A new growth of tissue in which the multiplication of cells is uncontrolled.
Neurocarcinogen	A substance which causes cancer in the tissues of the nervous system.
ng	nanogram, 10 ⁻⁹ of a gram.
NHL	Non-Hodgkins Lymphoma.
NOEL	No Observable Effect Level: The highest level, usually in food, at which symptoms of toxicity are not observed.
Non-Hodgkins Lymphoma	A malignant condition of the lymphatic system which does not possess the characteristics of Hodgkins Disease.
Nondisjunction	The failure of bivalent chromosomes to move apart during the process of cell division.
NTP	National Toxicology Program.
OR	Odds Ratio.

OSHA	Occupational Safety and Health Administration, of the U.S.
OSTP	Office of Science and Technology Policy, of the U.S.
Parenchymal	Referring to organs in the body cavity.
Paresis	Muscular weakness.
Percutaneous	Through the skin.
PGBE	Propylene Glycol Butyl Ether ester of 2,4-D.
pH	Acidity of a solution.
Pharmacokinetic	Referring to the distribution and movement of a chemical in an organism.
ppm	Parts per million.
Preneoplastic	Referring to events occurring before development of a tumor.
Prokaryotes	Organisms without a distinct nucleus.
Quadriparesis	Muscular weakness in all four limbs.
Radiolabelled	Containing a radioactive isotope.
Sister Chromatid	A chromatid from a homologous pair.
Sister Chromatid Exchange	Exchange of genetic material of sister chromatids as a result of genetic damage and as revealed by changes in banding patterns.
SCE	Sister Chromatid Exchange.
STS	Soft Tissue Sarcoma, a tumor of the connective tissues.
Subchronic	Toxicity studies where exposure is less than 0.25 of the lifespan.
TCDD	tetrachloro-p-dibenzodioxin.
Teratogenic	Causing developmental defects in the embryo or fetus.
Tryp.	tryptophan.
Tumor	A new growth of tissue in which the multiplication of cells is uncontrolled.
uCi	Unit of radioactivity.
UDS	Unscheduled DNA synthesis.
ug	microgram.

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